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สารจากนายกสมาคมเภสัชวิทยาแห่งประเทศไทย

เรียน สมาชิกสมาคมเภสัชวิทยาและผู้เข้าร่วมประชุมทุกท่าน

ในนามของสมาคมเภสัชวิทยาแห่งประเทศไทย ดิฉันขอต้อนรับทุกท่านเข้าร่วมประชุมวิชาการประจำปี ครั้งที่ 31 ด้วยความยินดียิ่ง

การประชุมวิชาการครั้งนี้ จัดขึ้นโดยความร่วมมือจาก ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ในการเป็นเจ้าภาพร่วม โดยมี รศ.ดร.วีรพล คู่คงวิริยะพันธุ์ เป็นประธานคณะกรรมการจัดการประชุม Theme ของการจัดประชุมครั้งนี้ คือ**From Basic to New Therapeutic Approaches** ซึ่งมีวัตถุประสงค์เพื่อเป็นการเชื่อมโยงให้เห็นความสัมพันธ์ชัดขึ้นระหว่างข้อมูลพื้นฐานทางเภสัชวิทยากับประโยชน์ในการนำไปใช้ทางคลินิก

ดิฉันหวังว่าทุกท่านจะได้รับประโยชน์ สมกับที่ท่านได้อุตส่าห์เดินทางมาไกล นอกจากความรู้ทางวิชาการแล้ว ฝ่ายจัดการประชุมยังมีกิจกรรมอื่นๆที่จะเป็นการเชื่อมความสมัครสมาน สามัคคีระหว่างสมาชิก หวังว่าทุกท่านจะเข้าร่วมในทุกกิจกรรมที่ได้เตรียมไว้ต้อนรับ โดยเฉพาะงานพาแลง ค่ำคืนนี้

ในนามของสมาคมฯ ดิฉันขอขอบคุณ วิทยากร คณะกรรมการจัดการประชุมฯ บริษัทห้างร้านที่ให้การสนับสนุน และท้ายสุดขอขอบคุณผู้เข้าร่วมประชุมทุกท่านที่มีส่วนร่วมในการทำให้ฝ่ายจัดการประชุมได้กำลังใจเป็นอย่างมาก ขอบคุณค่ะ

รศ ดร. จินดนา สัตยาศัย

นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

สารจากประธานจัดงานประชุมวิชาการประจำปีครั้งที่ 31

เรียน สมาชิกสมาคมเภสัชวิทยา ละผู้ร่วมประชุมทุกท่าน

ในนามของคณะกรรมการจัดการประชุม ผมขอขอบคุณทุกท่านที่ให้ความสนใจเข้าร่วมประชุมครั้งนี้ ที่มี Theme ของการประชุมว่า From Basic to New Therapeutic Approaches ซึ่งเราได้รับความกรุณาจากวิทยากรที่มีชื่อเสียงหลายท่าน กรุณามาบรรยายให้เราได้รับความรู้ใหม่ๆในด้านที่แต่ละท่านมีความเชี่ยวชาญ มีทั้งวิทยากรจากในประเทศและต่างประเทศ โดยเฉพาะอย่างยิ่ง Professor Edmund Jon Deoon Lee ที่จะเป็นองค์ปาฐก ในปาฐกถาพิเศษ รศ.จิรวัฒก์ สดาวงศ์วิวัฒน์ เรื่อง ABC Transporter: From Drug Resistance to Drug Response เป็นต้น

นอกจากการบรรยายเป็นเวลา 2 วันเต็มแล้ว ในปีนี้จะมี workshop อีก 1 วัน ในเรื่องที่กำลังเป็นที่สนใจ 1 เรื่อง คือ Pharmacogenomic of TPMT และเรื่องที่หวังว่าจะเป็นการช่วยฟื้นฟูการทำวิจัยด้วยสัตว์ทดลอง คือ workshop เรื่อง Integrative organ system Pharmacology Laboratory Hand on

ทั้งหมดนี้ ไม่ว่าจะเป็นการบรรยายใน 2 วันแรก และ workshop ใน 1 วันสุดท้าย ผมและทีมงานหวังว่าจะเป็นที่พอใจของทุกท่าน และทำให้ผู้เข้าประชุมทุกท่านได้รับความรู้ ทางวิชาการใหม่ๆ คุ้มค่ากับที่ท่านได้เสียสละเวลามาร่วมประชุม หากว่าการจัดประชุมครั้งนี้ ยังมีข้อต้องแก้ไข ปรับปรุงอย่างไร ผมและทีมงานขอน้อมรับคำเสนอแนะด้วยความขอบคุณเป็นอย่างยิ่ง

โอกาสนี้ ผมขอเรียนเชิญ ศาสตราจารย์เกียรติคุณ ดร.สุมนต์ สกลไชย อธิการบดีมหาวิทยาลัยขอนแก่น ได้กรุณากล่าวเปิดการประชุม ขอบคุณครับ

รศ.ดร. วีรพล คู่คงวิริยพันธุ์
ประธานกรรมการประชุมวิชาการฯ

คำปราศรัยของ ศาสตราจารย์เกียรติคุณ ดร. สุมนต์ สกลไชย อธิการบดี มหาวิทยาลัยขอนแก่น เนื่องในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 31 สมาคมเภสัชวิทยาแห่งประเทศไทย ในวันพุธที่ 18 มีนาคม 2552

ท่านนายกสมาคมเภสัชวิทยาแห่งประเทศไทย ท่านประธานคณะกรรมการจัดการประชุม และท่านผู้เข้าร่วมประชุมทุกท่าน

ผมรู้สึกเป็นเกียรติและยินดีเป็นอย่างยิ่งที่ได้มาเป็นประธานในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 31 ของสมาคมเภสัชวิทยาแห่งประเทศไทย ในวันนี้

เท่าที่ผมได้รับทราบจากสมาชิกของสมาคมฯที่อยู่ที่มหาวิทยาลัยขอนแก่นนี้ ได้ทราบว่าทางสมาคมฯสามารถจัดการประชุมวิชาการประจำปี ได้เป็นผลสำเร็จติดต่อกันมาได้ทุกปีจนถึงครั้งที่ 31นี้ นับว่าเป็นเรื่องที่น่ายินดีเป็นอย่างยิ่ง เพราะเป็นการทำให้สมาชิกจากทุกภาคพื้นของประเทศได้มีโอกาสรับทราบข้อมูลเชิงวิชาการทางเภสัชวิทยาใหม่ๆจากวิทยากรผู้ทรงความรู้ในด้านต่างๆ ทั้งในและนอกประเทศอย่างต่อเนื่อง

เป็นที่ทราบกันดีอยู่แล้วว่าในปัจจุบันวิชาการสาขาต่างๆมีข้อมูลเกิดใหม่อยู่ตลอดเวลา และเห็นชัดมากขึ้นทุกวันว่าความรู้ในแต่ละสาขานั้นล้วนมีความเชื่อมโยงถึงกัน การที่นักวิชาการในแต่ละด้านได้มีโอกาสมาประชุมร่วมกัน แลกเปลี่ยนความรู้ซึ่งกันและกันอย่างสม่ำเสมอ จึงนับว่าเป็นเรื่องที่น่ายินดีเป็นอย่างยิ่งว่าจะได้มีโอกาสร่วมมือกันในการสร้างผลงานทางวิชาการที่จะเป็นประโยชน์ต่อทุกฝ่ายและประเทศชาติ

ที่น่ายินดีอีกประการหนึ่งก็คือการที่ ปีนี้สมาคมฯให้โอกาสสมาชิกที่มหาวิทยาลัยขอนแก่นเป็นเจ้าภาพจัดการประชุม ทำให้มหาวิทยาลัยขอนแก่นเป็นที่รู้จักกันมากขึ้น ผมในนามของมหาวิทยาลัยขอนแก่นก็ขอร่วมแสดงความยินดี ต้อนรับผู้มาเยือน หวังว่าทุกท่านคงจะมีความสุขในการมาเยือนดินแดนแคว้นอิสานของเราในครั้งนี้

ผมขอใช้โอกาสนี้เปิดการประชุมวิชาการประจำปี ครั้งที่ 31 ของสมาคมเภสัชวิทยาแห่งประเทศไทย ขออวยพรให้การประชุมครั้งนี้สำเร็จตามวัตถุประสงค์ที่ตั้งไว้ทุกประการ

ศาสตราจารย์เกียรติคุณ ดร. สุมนต์ สกลไชย
อธิการบดีมหาวิทยาลัยขอนแก่น

บรรณาธิการแถลง

เรียนท่านสมาชิกสมาคมและผู้เข้าร่วมสัมมนา

เป็นที่น่ายินดีที่ได้มีโอกาสมาเยี่ยมเยือนสมาชิกชาวขอนแก่นอีกครั้ง Proceeding ของการประชุมวิชาการประจำปี ครั้งที่ 31 ของสมาคมเภสัชวิทยาแห่งประเทศไทยฉบับนี้เสร็จทันเวลาได้ จากความร่วมมืออย่างแข็งขันของทางเจ้าภาพฯ

ปีนี้มีผู้นำเสนอผลงานวิชาการมากมายกว่าทุกปี ส่วนใหญ่เป็นผลงานของวิทยานิพนธ์ของว่าที่วิทยาศาสตรมหาบัณฑิตจากหลักสูตร วท.ม สาขาวิชาเภสัชวิทยาจากทุกสำนักได้ส่งมา ทำให้เราได้ทราบความเป็นไปของสมาชิกที่อยู่กระจัดกระจายกันไปว่า มีแนวโน้มของงานวิจัยและมีความเจริญก้าวหน้าไปในทางใดบ้าง และอาจมีส่วนให้ได้ทำงานร่วมกันในอนาคต ที่น่าชื่นชมมากไปอีกก็คือนักเภสัชวิทยารุ่นใหม่ๆได้มีโอกาสมาพบปะสังสรรกันในบรรยากาศแบบกันเองฉันท์พี่ๆน้องๆ

อย่าลืมใช้เวลามีค่าของท่านในขอนแก่นอย่างคุ้มค่า ช่วยๆกันกระตุ้นเศรษฐกิจของจังหวัดด้วยการซื้อหาผ้าไหม ผ้าฝ้ายสวยๆ ของกินอร่อยๆ ติดไม้ติดมือไปฝากทางบ้าน ถ้ามีอะไรขาดตกบกพร่องก็มองข้ามๆไปมั่งก็ได้ คิดซะว่า ไม่มีอะไรสมบูรณ์แบบ ห้ามโกรธหรือเก็บไปหงุดหงิดนานเดี๋ยวจะเกิดความเครียดจากปฏิกริยาออกซิเดชั่น อาจจะแก่เร็วกว่าที่ควร หรือเป็นโรคซึมเศร้า ถ้าปลงยังไงก็ไม่ตกเสียที ให้ย้อนกลับไปอ่านเนื้อหาเรื่องเภสัชวิทยา-อารมย์ผิดปกติ ท่านได้นำเอาแนวคิดทางพุทธศาสนามาผสมผสานกับบทพิสูจน์อย่างวิทยาศาตร์ได้อย่างน่าเชื่อหรือไม่ ก็ลองติดตามดู

รศ.ดร.สุพัตรา ศรีไชยรัตน์

บรรณาธิการ

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ผศ.นพ. ดนุ เกษรศิริ	ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

กำหนดการประชุมวิชาการประจำปี ครั้งที่ 31

สมาคมเภสัชวิทยาแห่งประเทศไทย

ร่วมกับภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

วันที่ 18-20 มีนาคม 2552

ณ ห้องบรรยาย 1 คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Theme: From basic to new therapeutic approaches

วันพุธที่ 18 มีนาคม 2552

8:00-8:45 น.	ลงทะเบียน
8:45-9:00 น.	**ประธานกล่าวรายงาน** **พิธีเปิดการประชุมโดย อธิการบดี มหาวิทยาลัยขอนแก่น** นายกสมาคมฯ กล่าวต้อนรับผู้เข้าร่วมประชุม
9:00-9:45 น.	ปาฐกถานำ ดร.จิราวัฒก์ สดาวงศ์วงวิวัฒน์ **ABC Transporter: From Drug Resistance to Drug Response** **วิทยากร:** Prof. Edmund Jon Deoon Lee, Ph.D. Department of Pharmacology, National University of Singapore, Singapore
9:45-10:00 น.	พัก- อาหารว่าง
10:00-11:30 น.	**Session I: Targeted Drugs in Cancer Therapy** **วิทยากร:** รศ นพ ชวลิต ไพโรจน์กุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ผศ. พญ.เอื้อมเดือน สุขประเสริฐ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น **ผู้ดำเนินการอภิปราย:** ดร. เอื้อมเดือน ประวาฬ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
13:00-14:30	**Session II: From Neuroendocrine Control in Obesity to current Therapy** **วิทยากร:** รศ.พญ. ประณิธิ หงสประภาส ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น รศ.ดร.จุฑามณี สุทธิศรีสังข์ ภาควิชาเภสัชวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล **ผู้ดำเนินการอภิปราย:** ผศ.พญ.ศิริพร เทียมเก่า ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
14:30-14:45	พัก-อาหารว่าง
14:45-16:15	**Session III: Genetic Aspects of Immune-Mediated Adverse Drug Reactions** **วิทยากร:** รศ.นพ.เกื้อเกียรติ ประดิษฐ์พรศิลป์ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย รศ.ดร. ชาญวิทย์ ลีลายุวัฒน์ คณะเทคนิคการแพทย์ มหาวิทยาลัยขอนแก่น **ผู้ดำเนินการอภิปราย:** รศ.พญ. สุดา วรรณประสาท ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
16:15-17:00	**ประชุมสมาคมประจำปี**
19:00-21:00	**อาหารเย็น พานเลง แนะนำอาจารย์อาวุโส และการแสดง**

วันพฤหัสบดีที่ 19 มีนาคม 2552

8.30-9.15	**ปาฐกถารับเชิญ : Adolescent Depression: Adrenergic Mechanisms and Behavioral Correlates** **วิทยากร:** Prof. David B. Bylund, Ph.D. Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, USA
9.15-10.45	**Session IV: From Biology of Stem Cells to Clinical Application** **วิทยากร:** รศ.นพ.สุรเดช หงส์อิง ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล รศ.พญ.อริสา ลิมสุวรรณ ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล **ผู้ดำเนินการอภิปราย:** รศ. ดร. พิศมัย เหล่าภัทรเกษม ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
10.45-11.00	พักอาหารว่าง
11.00-12.00	**Session V: From Circadian Rhythms Impairment to Psychiatric Symptoms and Therapy** **วิทยากร:** ศ. นพ. อนันต์ ศรีเกียรติขจร ภาควิชาสรีรวิทยา, คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **ผู้ดำเนินการอภิปราย:** รศ ดร. จินดนา สัตยาศัย ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
12.00-13.00	**Lunch Symposium:** (Supported by Servier) **Neuroplasticity Approach For the Treatment of Depressive Disorders: Clinical Implications of Tianeptine** **วิทยากร:** Prof. S. Chattarji National Center for Biological Sciences, Bangalore, India
13.00-15.00	**Poster presentation:** ผู้นำเสนอผลงานประจำที่
15.00-15.15	พัก-อาหารว่าง
15.15-16.45	**Session VI: Pharmacology of Emotional Disorders** **วิทยากร:** รศ ดร.บพิตร กลางกัลยา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า นพ. ทวีศิลป์ วิษณุโยธิน ผู้อำนวยการสำนักสุขภาพจิตสังคม กรมสุขภาพจิต กระทรวงสาธารณสุข รศ.พญ.สุวรรณา อรุณพงศ์ไพศาล ภาควิชาจิตเวชศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น **ผู้ดำเนินการอภิปราย:** ผศ.นพ. คนุ เกษรศิริ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
16.45- 17.15	**พิธีมอบรางวัล Young Investigator Awards** **ปิดการประชุม**

วันศุกร์ที่ 20 มีนาคม 2552

8.30-12.00	**Work Shop group I**: **Pharmacogenomic of TPMT** บรรยาย : Principle of pharmacogenomics **Laboratory hand on:** รศ.พญ. สุดา วรรณประสาท และคณะ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
8.30-12.00	Lecture: Prof. David B. Bylund **Work Shop group 2: Integrative organ system pharmacology** **Laboratory hand on: Model studies of:** Neuromuscular junction, cardiovascular system *in vivo*, behavior studies, antianxiety, antidepressant, antipsychotic and learning & memory ผศ. ดร. ธารินี อรรควิเชียร และคณะ
12.00-13.00	**อาหารกลางวัน แล้ว เที่ยวในเมือง**: วัดหนองแวงพระธาตุ 9 ชั้น เยี่ยมโฮมบูนมัง ชมผ้าไหมพื้นเมือง
8.30-16.00	**Post-Conference Tour**: เยี่ยมชม พิพิธภัณฑ์ไดโนเสาร์ และผ้าพื้นเมือง ผ้าไหมแพรวา ที่จังหวัดกาฬสินธุ์

Chiravat Sadavongvivad Memorial Lecture

The ABC Drug Transporters : Their role in determining drug resistance and drug response

Edmund J.D. Lee
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Just over 30 years ago, Victor Ling described a membrane glycoprotein that mediated the efflux of drugs from Chinese hamster ovarian cells. Few would have recognized at that time, that this discovery would radically shape our understanding of drug response and toxicity. Today the glycoprotein has been identified as the multidrug resistance protein 1 (MDR1), and encoded by the ABCB1 gene on chromosome 7. Since the discovery of that P-glycoprotein, we now know that it is just one member of a large superfamily of ATP binding membrane transporters called ABC (ATP-binding cassette) transporters mediating efflux of various molecules across cell membranes. There are currently 48 recognized human ABC transporters represented by 7 families (ABCA-ABCG). These transporters are expressed almost ubiquitously in almost every cell type studied.

It is by now well understood that one of the critical functions of these ABC transporters is to protect the cell against the entry of foreign chemicals. When localized on the gastrointestinal mucosae, the action of ABC transporters reduces xenobiotic absorption into the body. At the tissue level, these transporters reduce penetration of unwanted chemicals into protected tissues such as the brain, testes and placenta etc. At the cellular level, ABC transporters protect cells against xenobiotic effects, as well as mediate the expulsion of metabolites generated by the cell.

Despite the progress made over the last 30 years, the understanding of the role that these transporters play in health and disease is still quite limited. The interactions of these transporters with xeno- and endobiotics create complex scenarios which continue to challenge the understanding of drug effects based on simplistic pharmacokinetic models.

Session I: Targeted Drugs in Cancer Therapy

Targeted Therapy: The Next Generation of Anticancer Drugs

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During the last decade, a better understanding of the biology of cancer cells has led to the identification of the genes, proteins, and pathways involved in cancer cell growth, survival, and metastasis. The therapies that target these specific characteristics of cancer cells have been developed, and these "targeted therapies" represent a new type of cancer treatment. This is one of the most rapidly expanding areas of clinical research because they hold the great promise of more effective therapies (increasing therapeutic power, higher ratcs of activity, and better rates of survival) with markedly fewer side effects. At present, several targeted agents, which include monoclonal antibodies and small molecule inhibitors, are already approved by the US-FDA for use in malignancies, and numerous others are currently in clinical trials.

Although targeted therapies are generally better tolerated than traditional chemotherapy, but they are not completely free from side effects. Additionally, the benefit to patients usually does not occur with dramatic tumor shrinkage rather it is with respect to overall survival and symptom control. Besides, they has raised several new questions for oncologists about the identification the subset of patients most likely to benefit from these specific drugs, the determination of optimal dosing and assessment of treatment effectiveness and toxicity, and the economics of cancer care. The present day, because of unperfected success of the targeted therapies, researchers agree that targeted therapies are not a replacement for traditional therapies, but an addition to adjuvant chemotherapy.

The next steps on the remarkable journey of further future targeted therapies are to accurately identify additional targets which having high specifically against cancer cells and the establishment of multi-targeted therapies through the combination of agents targeted to several distinct molecules. An increasing knowledge of targeted-based therapy offers promise that we will be able to make significant inroads in improving the clinical outcomes and the prognoses of cancer patients. Further investigations and the development of new drugs will be required to optimize the next generation of target-directed therapies.

Keywords: targeted therapy, anticancer drugs

Session I: Targeted Drugs in Cancer Therapy

Molecular Targeting for Chemoprevention and Therapy in Cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CC), a primary liver tumour that arises from biliary epithelial cells, and its incidence and mortality is high in NE -Thailand. The majority of CCs in this region are associated with chronic inflammation due to infection with liver flukes, Opisthorchis *viverrini*, in combination with nitrosamines from food ,however its molecular pathogenesis is still unknown.Prognosis of CC is poor as early surgical resection still remains the only curative treatment and currently no efficient secondary prevention or effective systemic treatments are available. However, in recent years, the emergence of target-based cancer therapies has provided the option of developing and testing novel treatment strategies . Endo *et al.*(2002) had demonstrated a strong positive correlation between levels of plasma membrane ErbB2 immunoreactivity and that of cytoplasmic cyclooxygenase-2 (COX-2) in related risk conditions and human CCs. The data generated from this quantitative immunohistochemical study are consistent with a growing body of experimental evidence suggests that an overexpression of the growth factor receptor tyrosine kinases c-ErbB-2, together with COX-2 signaling is implicated in cholangiocarcinogenesis. .In addition to activating through ErbB family receptors also activates other key cell regulatory molecules affecting cell differentiation, cell cycle progression, and malignant transformation and/or progression, including nuclear factor-kB (NF-kB), Cyclin D1(promotes G1/S phase cell cycle progression)the intracellular *ras-raf*-MEK-MAPK and PI3K-Akt cascades,interleukin-6/gp130, transmembrane mucins, hepatocyte growth factor/Met, and vascular endothelial growth factor signaling.The agents that selectively target ErbB receptors and related molecules having advanced the furthest in clinical development. However it is disappointing that the current clinical experience with ErbB-directed therapies have resulted in only very limited anti-tumor activity in CC . While it seems apparent that there is still a significant gap in our knowledge as how to best exploit such alterations in terms of targeted therapies. More definitive approaches, including cDNA microarray analysis, proteomics, may indicate the alternative modalities.

Session II: From Neuroendocrine Control in Obesity to current Therapy

Introduction to Obesity

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Introduction

Obesity is a chronic condition characterized by an excess of body fat and cause metabolic syndrome. It is often defined by the BMI (Body Mass Index), a mathematical formula that is highly correlated with body fat. BMI is expressed as weight in kilograms divided by height in metres squared (kg/m2). WHO classification of overweight and obesity is following by

< 18.5 kg/m2	= Underweight
18.5-24.9 kg/m2	= Normal range
25.0-29.9 kg/m2	= Overweight obese
30-34.9 kg/m 2	= Obese I
35-39.9 kg/m 2	= Obese II
>= 40 kg/m 2	= Obese III

In 1997, Aekplakorn, et al (1) report that 19.2 % of men and 33.9 % of women had BMI >25, whereas 3.5% of men 8.8 % of women of men had BMI >30. In obese children Thai Research, Sanguanrungsirikul ,et al (2) reported in 2001 that energy expenditure of obese children was higher than non-obese children because of greater body weight. Older age group, consumed more food during stressed, watching TV, VDO or playing computer continuously for more than 3 hours were significant associations with obesity.

Etiology and risk factors

Obesity is the result of long-term mismatches in energy balance and to develop when daily energy intake exceeds and physical activity is limited. Body weight is regulated by both hormonal and neuronal components. Hormonal signals include leptin, insulin, cortisol, ghrelin (from stomach), peptideYY and cholycystokinin (from small intestine) act through hypothalamus control centers and /via vagus nerve to release of hypothalamus peptides(neuropeptideY, Agouti-related peptide) for appetite stimulation. These regulation are intregrated with serotoninergic, catecholaminergic, endocannabinoid, and opioid signaling pathway. Genes (involving leptin), behavior, psychological, environment, culture, and socioeconomic status can influence appetite. Less commonly, obesity may also be induced by drugs (e.g. high-dose glucocorticoids), or be secondary to a variety of neuroendocrine disorders such as Cushing's syndrome and polycystic ovary syndrome.

Overweight and obese individuals are at increased risk for many diseases and health conditions, including the following:

- Stroke
- Depression
- Gallstone
- Dyslipidemia

- Type 2 diabetes
- Hypertension
- Coronary syndrome
- Sleep apnea and respiratory problems
- Joint and cutaneous disease: osteoarthritis, acanthosis nigricansReproductive problems (subfertility , complicated pregnancy, gestational diabetes, erectile dysfunction)

Treatment

The National Institutes of Health in the USA has issued guidelines for obesity treatment, which indicate that all obese adults (BMI greater than 30 kg/m2), and all adults with a BMI of 27 kg/m2 or more, and obesity-associated chronic diseases are candidates for drug treatment. Morbidly obese adults (BMI greater than 40 kg/m2) and all adults with a BMI of 35 kg/m2 or more, and obesity-associated chronic diseases are candidates for surgery. Goal of treatment is to improve comorbid conditions and reduce risk factors. Treatment consist of diet, physical activity, behavior therapy, pharmacotherapy and surgery. Maintaining the right energy balance, dietary modification (low –carbohydrate and fat, high-protein diet and daily fiber intake), 30 min of moderate intensity physical activity most days of the week and behavioral therapy such as stimulus control, self-monitoring techniques are more effectiveness for the treatment of obesity. Adjuvant pharmacologic treatment include central acting anorexiant medication (sibutramines), peripherally acting medication (oristat) and cannabinoid receptors antagonist.

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Clinical Pharmacolgy of Fosphenytoin

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Although phenytoin is well accepted to be effective in the treatment of epilepsy, it poses several limitations when given as intravenous (IV) loading doses such as poor aqueous solubility, incompatibility with saline and dextrose solutions. Intramuscular (IM) injection of parenteral solution of phenytoin is not recommended because it is erratically absorbed by this route and its solution is highly alkalinic (pH 12) leading to injection site reactions. In addition, its solution contains 40% propylene glycol and 10% ethanol resulting in adverse cardiovascular and central effects. Fosphenytoin, a prodrug of phenytoin has been developed to minimize these disadvantages. Fosphenytoin is readily soluble in water and is compatible with saline and dextrose solutions. It causes minimal injection site reactions and is rapidly converted by phosphatases in plasma to phenytoin. It is prescribed as phenytoin equivalence (PE) and no dose conversion is necessary. It can be infused at higher rates (50-150 mg PE/min) than IV phenytoin (50 mg/min) and hence shorter infusion time. The times to reach plasma therapeutic unbound and total phenytoin concentrations with 150 mg PE/min infusion of fosphenytoin are comparable to 50 mg/min infusion of phenytoin whereas infusion of fosphenytoin at the rate of 100 mg PE/min is matched with 30-40 mg/min infusion rate of phenytoin. IV fosphenytoin produces less cardiovascular complication but more pruritus and paresthesias than IV phenytoin. IV fosphenytoin is indicated for status epilepicus following IV diazepam or rectal diazepam for prolonged control of seizures. Fosphenytoin can also be injected intramuscularly since it is readily absorbed with bioavailabilty approaching 100% but it takes longer time to reach therapeutic levels than IV phenytoin or IV fosphenytoin. IM fosphenytoin offers advantages in non-emergent seizures or in situations where establishment of IV access is problematic or facilities for cardiac minitoring are not readily available. IM fosphenytoin may be safer than IV phenytoin or IV fosphenytoin in patients with existing cardiac disease or who are hemodynamically unstable. IM fosphenytoin rarely produces paresthesias or pruritus.

Current Management of Status Epilepticus

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Status epilepticus (SE) เป็นภาวะฉุกเฉินหนึ่งทางระบบประสาทก่อให้เกิดอัตราการเสียชีวิตที่สูงมาก ขึ้นกับสาเหตุ โรคประจำตัว ชนิดของ SE และการรักษาที่ถูกต้องรวดเร็วหรือไม่ การรักษาโรคลมชักในปัจจุบันมีความก้าวหน้าเป็นอย่างมาก ดังจะเห็นได้จากการประชุมวิชาการต่างๆ มีการบรรยายเกี่ยวกับ SE และยังมีการประชุมวิชาการเฉพาะเรื่อง SE อย่างเดียว ได้แก่การประชุม The London Colloquium on Status Epilepticus เมื่อ ปี พ.ศ. 2550 และมีการจัดทุก 2 ปี เพราะความรู้เกี่ยวกับ SE มีประเด็นต่างๆที่ต้องศึกษาอย่างมาก บทความนี้กล่าวเฉพาะนิยามของ SE แนวทางการรักษาของต่างประเทศ และของประเทศไทย

นิยาม

Epilepsy Foundation of America เมื่อปี พ.ศ. 2536 ได้ให้ข้อสังเกตว่านิยามเดิมของ SE คือการชักที่ต่อเนื่องนานมากกว่า 30 นาที หรือการชัก 2 ครั้งติดต่อกัน โดยระหว่างที่ผู้ป่วยหยุดชักนั้น ระดับความรู้สึกตัวไม่ฟื้นเป็นปกตินั้น นิยามดังกล่าวน่าจะก่อให้เกิดปัญหาความล่าช้าของการรักษา SE เพราะระยะเวลา 30 นาทีนั้นก่อให้เกิดภาวะ neuronal injury และ pharmacoresistance ดังนั้น Wasterlain และ Treiman จึงเสนอว่าควรลดระยะเวลาลงมาที่ 20 นาที 10 นาที หรือ 5 นาที และควรต้องพิจารณาลงไปในรายละเอียดด้วย เพราะระยะเวลาของการชักแต่ละชนิดก็มีระยะเวลานานไม่เท่ากัน เช่นการชักแบบ complex partial seizures (CPS) เฉลี่ยนาน 2.5 นาที การชักแบบ generalized tonic-clonic seizure (GTC) เฉลี่ยนาน 59.9 วินาที

ดังนั้นนิยามของ SE แบบ GTC นั้นควรกำหนดที่ระยะเวลานาน 5 นาที และเมื่อพิจารณาในรายละเอียดของ SE แล้ว ประกอบด้วย 3 ระยะ ได้แก่

1. Impending SE คือระยะเวลาชักนานมากกว่า 5 นาที ซึ่งต้องรีบให้การรักษาแบบ SE เพื่อป้องกันไม่ให้เกิดภาวะ neuronal injury และ pharmacoresistance ตามมา
2. Established SE คือระยะเวลาชักนานมากกว่า 30 นาที โดยผู้ป่วยหมดสติตลอด ยิ่งต้องรีบให้การรักษาอย่างรวดเร็วที่สุด เพื่อป้องกันไม่ให้เป็น refractory SE
3. Subtle SE คือ ระยะสุดท้าย หรือ burned-out ของ SE เพราะผู้ป่วยมีอาการทาง motor น้อยมาก แต่ epileptic activity ในสมองมีตลอดเวลา

SE เกิดขึ้นได้อย่างไร

ปกติแล้วเซลล์สมองจะมีกลไกในการยับยั้งไม่ให้เกิดการชักอย่างต่อเนื่อง ดังจะเห็นได้จากการชักนั้นจะหยุดได้เอง โดยที่ไม่จำเป็นต้องให้ยา แต่ SE นั้นเกิดภาวะ electrical stimulation ทั่วทั้งสมองส่งผลให้มี chemoconvulsants กระจายไปทั่วทั้งสมอง ส่งผลให้กระบวนการยับยั้งนั้นไม่สามารถชนะกระบวนการกระตุ้นให้เกิดการชักได้ หรือกล่าวอีกนัยหนึ่ง Gamma-Aminobutyric Acid (GABA) ซึ่งเป็นสารยับยั้งนั้นมีประสิทธิภาพด้อยกว่า Glutamate

ตารางที่ 1 แสดงถึงสารที่เป็นตัวเริ่มต้นและสารที่จะใช้ยับยั้งกระบวนการเกิด SE

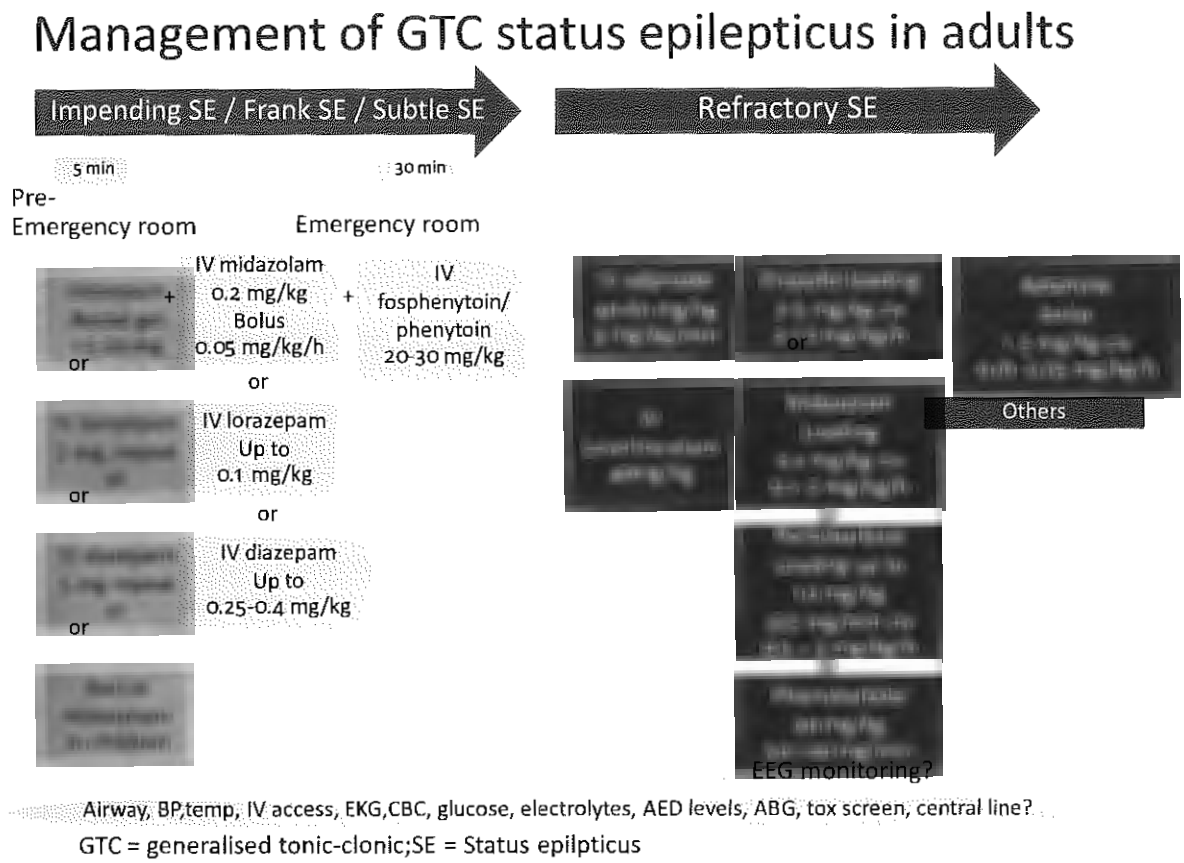
Mechanism and Treatment of Status Epilepticus

Initiation and maintenance of SE		
Initiators	Blockers of initiation phase	Blockers of Maintenance phase
Low Na^+, High K^+	*Na^+ channel blockers*	NMDA antagonists
$GABA_A$ antagonists	*$GABA_A$ agonists*	Tachykinin antagonists
Glutamate agonists:NMDA, AMPA,kainate, low Mg^{2+}, low Ca^{2+}, stimulation of glutamatergic pathways	*NMDA antagonists, high $Mg^{2+}{}_o$* *AMPA/kainate antagonists*	Galanin
Cholinergic muscarinic agonists, stimulation of muscarinic pathways	*Cholinergic muscarinic antagonists*	Dynorphin
Tachykinins (SP,NKB)	*SP,Neurokinin B antagonists*	
Galanin antagonists	*Galanin*	
Opiate δ agonists	*Somatostatin*	
Opiate *k* antagonists	*NPY*	
	Opiate δ antagonists	
	Dynorphin (k agonist)	

AMPA, Aα-amino-3-hydroxy-5-methylisoxazolc-4-propionic acid; GABA,gamma-aminobutyric acid; NKB, neurokinin B; NMDA, N-methyl-D-aspartate;NPY,neuropeptide Y;SP,substance P.

แนวทางการรักษา SE

จากการประชุม The London Colloquium on Status Epilepticus ที่ประเทศอังกฤษ แพทย์ผู้เชี่ยวชาญได้พิจารณาถึงแนวทางเดิม ประสิทธิภาพของยาใหม่ และความพร้อมของโรงพยาบาลต่างๆ ได้สรุปแนวทางการรักษาเป็น 3 ระยะคือ 1. Pre-hospital 2. Emergency room และ 3. Intensive care unit รายละเอียดดังภาพที่ 1



แนวทางการรักษา SE ประเทศไทย

หลักการรักษาที่สำคัญและต้องปฏิบัติพร้อมกันใน 4 ด้านคือ

1.หยุดอาการชักให้เร็วที่สุด

2.ป้องกันการชัก

3.รักษาสาเหตุ

4.ป้องกันและรักษาภาวะแทรกซ้อน

แบ่งการรักษาเป็น 2 ระยะ คือ

1. การรักษาเบื้องต้นก่อนมาถึงโรงพยาบาล

- เปิดทางเดินหายใจให้โล่ง โดยการจัดผู้ป่วยให้อยู่ในท่านอนตะแคง นำอาหารหรือฟัน
- ปลอมที่มีอยู่ในปากออก และปลดเสื้อผ้าที่รัดให้ผู้ป่วยหายใจได้สะดวก
- จัดให้ป่วยนอนอยู่ในบริเวณที่ปลอดภัย ป้องกันอันตรายจากการกระแทกกับของแข็ง
- ห้ามใช้ไม้กดลิ้นหรือวัตถุใดๆ สอดเข้าไปในปากหรืองัดปากผู้ป่วยขณะชักเกร็งกัดฟัน
- เพราะอาจจะเกิดอันตรายจากฟันหักตกลงไปอุดหลอดลมได้
- รีบนำส่งสถานพยาบาลหรือโรงพยาบาลใกล้บ้านให้เร็วที่สุด

2. การรักษาในสถานพยาบาลหรือรถฉุกเฉิน

1. เปิดทางเดินหายใจให้โล่ง โดยการจัดผู้ป่วยให้อยู่ในท่านอนตะแคง นำอาหารหรือฟัน ปลอมที่มีอยู่ในปากออก และปลดเสื้อผ้าที่รัดให้ผู้ป่วยหายใจได้สะดวก

2. ส่งเลือดตรวจหาระดับ glucose, electrolytes, calcium, magnesium และตรวจนับเม็ดเลือดให้พิจารณาเป็นรายๆ ควรเก็บเลือดบางส่วนไว้สำหรับตรวจหาสารพิษ เพาะเชื้อ ระดับยากัน

ชัก และอื่น ๆ ที่อาจจะมีข้อบ่งชี้ภายหลัง

3. ควรให้สาร glucose, maintenance fluid และวิตามินบี ดังนี้

3.1 ผู้ป่วยเด็กให้ 25% glucose ขนาด 2 มล./กก. และให้ 5% dextrose in saline (ชนิดของสารน้ำพิจารณาตามความเหมาะสม) เด็กที่อายุต่ำกว่า 18 เดือนให้ pyridoxine (Vitamin B6) 100 มก. ทางหลอดเลือดดำ

3.2 ผู้ป่วยผู้ใหญ่ให้ 50% glucose 50 มล. รวมกับ thiamine (Vitamin B1) 100 มก. ทางหลอดเลือดดำ และให้ normal saline solution

4. ประเมินผู้ป่วยอย่างรวดเร็วถึงชนิดของอาการชักและสาเหตุ

5. ให้ยากันชักที่ออกฤทธิ์เร็ว ได้แก่ diazepam ขนาด 0.3 มก./กก. ทางหลอดเลือดดำ หรือ 0.5 มก./กก. ทางทวารหนักในเด็ก หรือ 10 มก. ทางหลอดเลือดดำในผู้ใหญ่ และถ้ายังชักอยู่อาจให้ซ้ำในขนาดเดียวกันภายใน 15 นาที (ไม่ควรเกิน 2 ครั้ง) เนื่องจากฤทธิ์ของยา diazepam อยู่ได้นานเพียง 15 นาที ดังนั้นต้องให้ยากันชักที่ออกฤทธิ์นานตามด้วยทันที ได้แก่

5.1 Phenytoin ขนาด 20 มก./กก. ผสมในน้ำเกลือที่ไม่มีกลูโคส ให้ทางหลอดเลือดดำ ด้วยความเร็วไม่เกิน 1 มก./กก./นาทีในเด็ก หรือไม่เกิน 50 มก./นาที ในผู้ใหญ่ ในระหว่างที่ใช้ยานี้ควรดิดตามการเต้นของหัวใจและความดันโลหิต ถ้ายังมีอาการชักอีกอาจให้ยาซ้ำ ในขนาด 10 มก./กก. (ขนาดยารวมไม่เกิน 30 มก./กก.)

5.2 กรณีไม่มีหรือไม่ดอบสนองต่อ phenytoin ให้ phenobarbital ขนาด 20 มก./กก. ผสมในน้ำเกลือให้ทางหลอดเลือดดำ ด้วยความเร็วไม่เกิน 2 มก./กก./นาที ในเด็ก หรือไม่เกิน 100 มก./นาที ในผู้ใหญ่ ในระหว่างการให้ยาควรติดตามการหายใจ ถ้ายังคงชักอยู่ให้ยาซ้ำได้ในขนาด 10 มก./กก. หากยังไม่หยุดชักให้ดูรายละเอียดตามข้อ 6.

6. การดำเนินการรักษาดังกล่าวข้างต้น ถ้ายังไม่หยุดชักใน 60 นาที ให้ถือว่าผู้ป่วยอยู่ในภาวะชักต่อเนื่องชนิดไม่ตอบสนองต่อการรักษา (refractory status epilepticus) ควรพิจารณาย้ายผู้ป่วยเข้า ICU เพื่อติดตาม vital signs ถ้ามีเครื่องมือพร้อมควรติดตามดูคลื่นไฟฟ้าหัวใจ oxygen saturation คลื่นไฟฟ้าสมอง และดำเนินการให้ยารักษาโดยเลือกใช้ยาชนิดใดชนิดหนึ่งต่อไปนี้

6.1 Pentobarbital ขนาด 5-20 มก./กก. โดยให้ทางหลอดเลือดดำในอัตราไม่เกิน 25 มก./นาที ตามด้วย 0.5-1 มก./กก./ชม. สามารถเพิ่มถึงขนาด 3 มก./กก./ชม. จนกว่าจะหยุดชักหรือคลื่นไฟฟ้าสมองเป็นแบบ burst suppression

6.2 Thiopental ขนาด 100-250 มก. ทางหลอดเลือดดำตามด้วย 50 มก. ทุก 2-3 นาที จนกว่าจะหยุดชักหรือคลื่นไฟฟ้าสมองเป็นแบบ burst suppression หลังจากนั้นให้ยาขนาด 3-5 มก./กก./ชม.

6.3 Propofol 1-3 มก./กก. ทางหลอดเลือดดำ ตามด้วย 5-10 มก./กก./ชม. จนหยุดชักหรือมีคลื่นไฟฟ้าสมองเป็นแบบ burst suppression แล้วลดขนาดเป็น 1-3 มก./กก./ชม.

6.4 Midazolam 0.1-0.3 มก./กก. ทางหลอดเลือดดำ ตามด้วย 0.05-0.4 มก./กก./ชม. ระหว่างให้ยาตังข้อ 6 ต้องให้ยากันชักพื้นฐานอย่างน้อย 1 ชนิด ในขนาด maintenance ต่อไปเมื่อหยุดชักนานอย่าง

น้อย 24-48 ชั่วโมง ให้พิจารณาลดขนาดยาหยดทางหลอดเลือดดังข้อ 6 ลงอย่างช้า ๆ จนหยุดยาในเวลา 12-24 ชั่วโมง ถ้ามีการชักซ้ำใหม่ให้เพิ่มขนาดของยาที่รักษาได้ผล

7. **ระหว่างให้การรักษาอาการชักควรสาเหตุ และทำการแก้ไขไปพร้อมกัน** สาเหตุที่พบบ่อย ได้แก่ การขาดยากันชัก การติดเชื้อ สาเหตุทางเมตาบอลิก การได้รับยาและสารพิษ การบาดเจ็บที่ศีรษะ เป็นต้น
8. **ควรเฝ้าระวังและแก้ไขภาวะแทรกซ้อนที่อาจเกิดขึ้น** ได้แก่ metabolic acidosis, electrolyte imbalance, hypoxia ภาวะสมองบวม ปอดอักเสบจากการสำลัก เป็นต้น

หมายเหตุ

1. การให้ diazepam ทางทวารหนักในเด็ก ให้ใช้ diazepam ชนิดฉีดเข้าเส้น โดยใช้ insulin syringe แบบ plastic หรือต่อสายสวนทางทวารหนักสอดลึกประมาณ 2 นิ้ว ต้องยกก้นและหนีบรูทวารผู้ป่วยประมาณ 2 นาที เพื่อให้ยาไม่ไหลออก
2. ในกรณีที่ไม่มียา phenytoin และ phenobarbital สามารถใช้ sodium valproate โดยการฉีดเข้าหลอดเลือดดำได้ขนาด 15-20 มก./กก. ในอัตรา 1.5-3 มก./กก./นาที หลังจากนั้นหยดต่อในอัตรา 1 มก./กก./ชม.
3. แนวทางการรักษาอาการชักต่อเนื่องนี้ไม่ใช่สำหรับ neonatal seizure
4. สำหรับ non-convulsive epilepticus ที่ไม่ใช่ absence status epilepticus ยังไม่มีข้อสรุปสำหรับแนวทางการรักษาที่แน่ชัด
5. กรณี absence status epilepticus สามารถปรับขนาดยา sodium valproate และติดตามอาการ
6. กรณี epilepsia partialis continua การรักษาควรพิจารณาเป็นกรณีไปโดยพิจารณาถึงผลประโยชน์จากการควบคุมการชักเทียบกับผลข้างเคียงจากยากันชัก

วิตามินบีรวม* ชนิดฉีดเข้าเส้น (MIMS Thailand 1/2003)

1. BECOLIM 100®/ml (Atlantic Lab) Vit. B1 100 mg, Bit. B2 2 mg, Vit. B6 10 mg. IM/IV, niacinamide 100 mg, d-pantothenol 1 mg. (IM/IV)
2. STAMINA®/ml (S.charoen Bhaesaj) 100 ml Vit. B1 2.5 mg, Vit. B2 2 mg, Vit. B6 2 mg, niacinemide 25 mg, dextrose monohydrate 5 g. (IV)
3. B-100 complex/ml (TP Drug) Vit. B1 100 mg, Vit. B2 0.5 mg, Vit. B6 1 mg, nicotinamide 100 mg, (IM/IV)
4. Douzabox injection/3 ml (Sriprasit Dispensary) Vit. B1 100 mg, Vit. B6 100 mg, Vit. V12 1000 Mcg (IM)
5. Genavit/ml (Generl Drug House) Vit. B1 100 mg, Vit. B6 50 mg, Vit. B12 1000 mcg (IM)
6. Metaplex/ml (Takeda Zuellig) Vit. B1 100 mg, Vit. B2 5 mg, Vit. B6 5 mg nicotinamide 50 mg, pantothenol 5 mg (IM/IV)

อย่างไรก็ตามจากประสบการณ์การดูแลผู้ป่วย SE มาเกือบ 20 ปี พบว่าปัญหาสำคัญของการรักษาคือ

1. การให้การวินิจฉัยที่ล่าช้า
2. ขาดการเฝ้าดูแลรักษาผู้ป่วยอย่างต่อเนื่อง และ
3. การบริหารยาที่ผิดพลาด

ทั้ง 3 ปัจจัยที่ส่งผลให้การรักษา SE ไม่ประสบผลสำเร็จ

ตารางที่ 2 การปฏิบัติในการให้การรักษาภาวะชักต่อเนื่องในโรงพยาบาล

Stage of status	General measures	AED treatment
Premonitory (0-10 minutes)	ประเมินการทำงานของระบบหายใจ และการไหลเวียนโลหิต เปิดทางเดินหายใจให้ออกซิเจน ประเมินการชัก	Diazepam (i.v.bolus or p.r.)
Early (10-30 minutes)	ติดตามสัญญาณชีพ และการชัก เปิดหลอดเลือดดำ เจาะเลือดส่งตรวจ ให้ 50% glucose 50 ml และวิตามินบี 1 หรือวิตามินบีรวม * ในเด็กให้ 25% glucose ขนาด 2 มล./กก. ถ้าอายุต่ำกว่า 18 เดือนให้วิตามินบี 6 100 มก. ส่งต่อไปยังหอผู้ป่วยหนัก (ถ้ายังชัก)	Diazepam (i.v.bolus) ตามด้วย phenytoin (i.v.loading) หรือ phenobarbital (i.v.loading) หรือ sodium valproate (i.v.loading) ถ้าไม่มียา phenytoin หรือ phenobarbital
Established (30-60 minutes)	ค้นหาสาเหตุและรักษาภาวะแทรกซ้อนอาจให้ยาช่วยเพิ่มความดันหากจำเป็น	ถ้ายังชักอยู่ใช้ยาเดิม (phenytoin หรือ phenobarbital) ขนาดครึ่งหนึ่งของครั้งแรก ยังไม่หยุดชักเลือกยาอีกชนิดหนึ่ง phenobarbital (i.v.loading) หรือ phenytoin (i.v.loading) หรือ sodium valproate (i.v.loading) ถ้าไม่มียา phenytoin หรือ phenobaribital
Refractory (>60minutes)	เฝ้าติดตามสัญญาณ EEG, ติดตามการชักและการทำงานของระบบประสาท	Propofol (i.v.bolus & inf) midazolam (i.v.bolus) pentobarbital (i.v.bolus & inf) หรือ thiopental (i.v.bulus & inf)
i.v.=intravenous, p.r.=per rectum, i.m.=intramuscular, inf=infusion		

สรุป ภาวะ SE เป็นภาวะที่แพทย์ต้องให้ความตระหนักและต้องเฝ้าติดตามผู้ป่วยอย่างต่อเนื่อง การหยุดชักได้เร็วที่สุดเท่าไร ก็จะส่งผลดีต่อผู้ป่วยมากที่สุดเช่นกัน ดังนั้นยากันชักที่สามารถให้ได้อย่างรวดเร็วก็จะมีประโยชน์อย่างยิ่ง

บรรณานุกรม

1. แนวทางการรักษาโรคลมชัก สมาคมโรคลมชักแห่งประเทศไทย พ.ศ.2550
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Session III: Genetic Aspects of Immune-Mediated Adverse Drug Reactions

The Association of Anti-r-HuEpo Associated Pure Red Cell Aplasia with HLA-DRB1*09-DQB1*0309

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Abstract

Recombinant human erythropoietin (r-HuEpo) has been used to improve morbidity and mortality in chronic kidney disease. Subcutaneous use of r-HuEpo causes immunogenicity and develops anti-r-HuEpo antibody which has been demonstrated as the cause of anti-r-HuEpo associated pure red cell aplasia (PRCA). There are no known risk factors to predict the development of subcutaneous used anti-r-HuEpo associated PRCA. Our data demonstrated the association of HLA in anti-r-HuEpo associated PRCA patients. The distribution of gene frequency of HLA-A, -B, -DR and -DQ alleles in anti-r-HuEpo associated PRCA cases showed high gene frequency of HLA-A* 02, HLA-A* 11 and HLA-A*24 for HLA-A loci, HLA-B*18, HLA-B*46, HLA-B*60 and HLA-B*62 for HLA-B loci, and HLA-DRB1*09, HLA-DRB1*12 and HLA-DRB1*15 for HLA-DR loci. Compared with HLA gene frequency of the potential cadaveric kidney transplantation recipients in the waiting list and the potential bone marrow stem cell donors in national stem cell registry, there was significant difference of HLA-DRB1*09 HLA-DQB1*0309 ($p < 0.001$) gene frequency in anti-r-HuEpo associated PRCA cases. The odd ratio of HLA-DRB1*09 allele for anti-r-HuEpo associated PRCA was 2.89 (95% CI: 1.88-4.46; p-value: <0.001) Our data indicated the contribution of HLA genotype to the genetic susceptibility to anti-r-HuEPO associated PRCA. The HLA-DRB1*09-DQB1*0309 showed the association with anti-r-HuEpo associated PRCA and may be used in identifying the risk of the patients.

Introduction;

Recombinant human erythropoietin (r-HuEpo) has been used in clinical practice since late 1980s to improve anemia caused by erythropoietin deficiency in chronic kidney disease. Although r-HuEpo improves mortality and morbidity associated with anemia of the patients, the adverse immunological effect of this agent has been reported. After the introduction of r-HuEpo, sporadic cases of anti-r-HuEpo antibody developed with the subcutaneous administration have been identified (1-3). Subsequently, thirteen patients with chronic kidney disease, in whom loss of r-HuEpo efficacy and blood transfusion-dependent anemia occurred due to pure red cell aplasia (PRCA) after an initial response to subcutaneous use of r-HuEpo have been studied. Anti-r-HuEpo has been demonstrated as the proximate cause of PRCA (4). Patients with anti-r-HuEpo associated PRCA suddenly experience a loss of efficacy of treatment and simultaneously have decreases in hemoglobin and circulating reticulocyte count. The bone marrow biopsy from these patients showed an absence of erythroid precursor cells with normal cell population of non-erythroid lineage. The anemia in patients who had anti-r-HuEpo associated PRCA is more severe than anemia of chronic kidney disease at the same stage of disease, indicating the cross reactivity between r-HuEpo and native erythropoietin by the homology of the molecule. The anti-r-HuEpo antibodies are directed against the peptide of r-HuEpo molecule rather than the carbohydrate moiety and cause loss of r-HuEpo efficacy by the neutralizing effect. The established risk factor for anti-r-Hu-Epo associated PRCA included subcutaneous administration (5) and the presence of leachates

from uncoated stoppers in formulation containing polysorbate 80 (6). Theoretically, the break of tolerance by repeated subcutaneous injection of r-HuEpo initiated by the allorecognition involves the major histocompatibility. Previous observation of our report
showed an aggregation of HLA-DRB1*09 in four anti-r-HuEpo associated PRCA cases (7). This allele is rare in Caucasian (< 1%) but more common in Thai population (8.4–12.5%) (8). Previous studies have shown HLA genes influence immune response in relation to drug treatment (9-10).

Clinical diagnosis of anti-r-HuEpo associated pure red cell aplasia:

The essential features (11-12) for the diagnosis of anti-r-HuEpo associated PRCA included 1) sudden onset of loss of efficacy of r-HuEpo, 2) low reticulocyte count (below 10 X 109/L), and 3) positive for anti-r-HuEpo antibody by radioimmunoprecipitation, using of 125Iodinated erythropoietin (13-14), and 4) bone marrow biopsy showed normocellularity and absence of erythroid precursor (< 5% of erythroblast in bone marrow) with normal myeloid and megakaryocytic lineages. The CFU-E bioassay hs been used to confirm the neutralizing effect towards erythroid precursor.

Role of HLA in anti-r-HuEpo associated pure red cell aplasia:

Repeated subcutaneous injection of r-HuEpo pharmacologic doses over an extended period of time induces immune response. The induction of an immune response to r-HuEpo results in an autoimmune reaction to the endogenous native form. Although
there are evidences that other biopharmaceutical agents namely: G-CSF, GM-CSF, interferon α, interferon β has potential immunogenicity as antibodies to the recombinant protein developed in patients using the agents have been reported (15), only the neutralization effect of anti-r-HuEpo antibody which crosses react with the native molecule has been demonstrated. Our data showed that most of anti-r-HuEpo associated PRCA cases were diagnosed by the time of using alpha r-HuEpo, yet the use of beta r-HuEpo did not exclude the possibility of the condition. Recently, there is a leachate theory which explains the release of the organic compound leached from the exposure of polysorbate-80 as preservative to the uncoated stoppers in prefilled syringes as the cause of the immunogenicity and the use of Teflon coated stopper prefilled syringes of r-HuEpo reduces the risk of anti-r-HuEpo antibody in European country. Our data showed that yet the use of Teflon-coated stopper pre-filled syringe which was free from leachate did not exclude risk of the neutralizing antibody as there were cases received the coated stopper during the diagnosis of anti-r-HuEpo associated PRCA and this disparity of the prevalence between ours and European population might be explained by HLA genetic polymorphism.

By typing the HLA alleles expressed by individual anti-r-HuEpo associated PRCA cases compared to the HLA alleles of potential kidney transplant recipients in the waiting list, which represent chronic kidney disease population, and HLA alleles of national stem cell donor registry, which represent normal healthy population, there was an association between the HLA-DRB1*09 and the risk of anti-r-HuEpo associated PRCA. The presence of HLA-DRB1*09 in anti-r-HuEpo associated PRCA linked with HLA-DQB1*0309 (HLA-DRB1*09-DQB1*0309) as all PRCA cases who had HLA-DRB1*09 also had HLA-DQB1*0309. This finding demonstrated the genetic linkage between these two HLA alleles. The non-statistical significant difference of genotypic HLA alleles between potential kidney transplant recipient and national stem cell donor allowed us to conclude that the higher specific gene frequencies in anti-r-HuEpo associated PRCA cases were not caused by the chronic kidney disease genetic risk of the population. Recent case-control study of the

association between HLA gene and anti-r-HuEpo associated PRCA in another set of population mainly Caucasian in Europe and Canada concurred with our findings as HLA-DR B1*9 occurred at a significant higher frequency in antibody-positive PRCA case compared to control (16).

Association of HLA and autoimmune diseases is well known and is widely studied across the population worldwide and are found to be important in prediction of disease susceptibility. Also the specific HLA has been shown to associate with drug hypersensitivity which demonstrated the significance of HLA in immune allo-recognition. process. Analysis of population-specific distribution of HLA alleles is proved to be important in finding out disease susceptibility or resistance in specific ethnic groups.

Different population tends to exhibit frequency distributions of alleles. A recent study showed the association between HLA-DRB1*09 and DQ-B1*0303 and type 1 diabetes mellitus (17) in Asian population. Our finding of the association of HLA-DRB1*09-DQB1*0309 and anti-r-HuEpo associated PRCA may explain why there are more prevalence of anti-r-HuEpo associated PRCA cases in our population compared with Caucasian as this allele is rare in Caucasian.. The concept of specificities of HLA to involve in selection of potentially autoreactive T-cell specificities, peripheral amplification of the potentially autoreactive T-cells to r-HuEpo which cross react to native erythropoietin molecule need further study both in another set of population where the frequency of HLA-DRB1*09 are comparable.

In summary, the anti-r-HuEpo antibody developed after subcutaneous injection of r-HuEpo exerted the potential immunogenicity of biopharmaceutical agent which was related to specific HLA for the allorecognition process. Our data indicated the contribution of HLA genotype to the genetic susceptibility to anti-r-HuEPO associated PRCA. The HLA-DRB1*09-DQB1*0309 showed the association with anti-r-HuEpo associated PRCA and may be used in identifying the risk of the patients.

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Role of Human Leukocyte Antigen (HLA) in Immune Responses: Relevance to Immune Mediated Adverse Drug Effects (IMADE)

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Abstract

Human leukocyte antigens (HLA) or human major histocompatibility complex (MHC) are key molecules in immune responses. They present peptide antigens to T cells generating adaptive immunity by interaction with specific T cell receptors (TCR). There are two classes of MHC molecules; MHC class I and II. Classical MHC class I consists of HLA-A, HLA-B and HLA-Cw. These molecules are expressed on most nucleated cells acting as target for cytotoxic CD8+ T cells to probe for abnormal or foreign peptide antigens in the cells leading to cell killing. Classical MHC class II includes HLA-DR, HLA-DQ and HLA-DP. These molecules are expressed only on antigen presenting cells or inducible in activated immune cells. Thus, MHC class II plus peptide interaction with specific TCR on CD4+ T cells leading to immune stimulation/activation and propagation of effector T cells both cytokine producing and cytotoxic T cells generating specific immune responses. In addition to cellular mediated immune responses, these T cells are also involved in B cell activation and production of antibodies especially in isotype switching from IgM to IgG, IgA and IgE which is required in hypersensitivity. Thus, MHC is involved in immune mediated drug effects both type I and type IV which are IgE mediated and T cell-mediated delayed type hypersensitivity. Recently, a concept of pharmacological interaction of drugs with immune receptors or "the p-I concept" has been proposed leading to adverse drug effects mediated by immediate T cell responses but not a delayed type. Several strong MHC associations with adverse drug effects have been reported such as *HLA-B*1502* and antiepileptic carbamazepine, *HLA-B*57* and the reverse transcriptase inhibitor abacavir as well as *HLA-B*58* and the gout prophylactic allopurinol. It is well known that HLA molecules are highly polymorphic with hundreds of alleles. These polymorphisms affect a peptide binding repertoire leading to diverse immune activation. Interestingly, there are limited numbers of common alleles in each population. For example, only four major HLA-A alleles (*HLA-A*02* (26%), *A*11*(23%), *A*24*(19%) and *A*33*(12%)) cover 80% of the northeastern Thai (NET) population. Similarly, 8 major HLA-B alleles, 5 alleles of HLA-Cw, 6 alleles of HLA-DRB1 and 5 alleles of HLA-DQB1 cover approximately 80% of the population. The most common HLA-B allele in NET is *HLA-B*15* which forms a large group of *HLA-B*15* subtypes. Of these, *HLA-B*1502* is the most common found in 64.3% of the *HLA-B*15* positive NET individuals. This presentation provide information on HLA polymorphism in northeastern Thais that can be used for further studies of HLA associations and immune mechanisms involved in immune mediated adverse drug effects.

Keyword: HLA, polymorphism, immune-mediated adverse drug effects

Invited Lecture

Adolescent Depression: Adrenergic Mechanisms and Behavioral Correlates

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The pharmacological treatment of depression in children and adolescents is difficult due to a lack of effective treatments for this age group. Many of the antidepressants used to treat adult depression cannot be used for pediatric depression because of a lack of efficacy and/or side effects. Specifically the tricyclic antidepressants show good efficacy in the treatment of adult depression, but are no better than placebo in treating pediatric depression. In order to better understand differential response of children and adolescents, as compared to adults, to antidepressant drugs, it is useful to compare juvenile rats with adult rats. Recent studies have shown both neurochemical and behavioral differences between adult and juvenile animals after antidepressant treatment. In addition, juvenile animals have differences as compared to adult animals in the maturation of the serotonergic and noradrenergic systems, and in the dose of antidepressant drug needed to achieve similar brain levels. Differences after administration of antidepressant drugs have also been reported for adrenergic receptor regulation, a physiologic hypothermic response, as well as behavioral differences in two animal models of depression. A better understanding of the effects of antidepressants in juvenile animals should be beneficial for studying and finding new treatments for pediatric depression.

Major depressive disorder is a debilitating and serious mental illness that affects approximately two to five percent of the population worldwide, with a lifetime prevalence of around 15%. The World Health Organization projects that by the year 2020, depression will be second only to heart disease as the leading cause of disability worldwide. Although the clinical symptoms of adolescent and childhood depression vary with developmental age, overall they are similar to those seen in adults. Depression is one of the most common mental health disorders in the pediatric population, with a prevalence in children of up to 2.5% and during adolescence somewhere between 4% to 8%.

In adults, depression can be successfully treated with several classes of antidepressant drugs including tricyclic antidepressants (TCA; desipramnine, imipramine), selective serotonin reuptake inhibitors (SSRI; fluoxetine, citalopram), selective norepinephrine reuptake inhibitors (NRI; reboxetine) and serotonin norepinephrine reuptake inhibitors (SNRI; venlafaxine). The classes do not differ significantly in their effectiveness, although there are some differences among individual drugs (1). They do differ in their side effect profile. The important advantage of the SSRIs over the TCAs is not their selectivity for the serotonin transporter over the norepinephrine transporter, but their lower affinity for the biogenic amine receptors mediating the adverse effects (2). The currently available evidence suggests that the initial step in the mechanism of action of these antidepressants is an increase in monoamine levels. Thus, it appears that other adaptive changes, such as those in G protein-coupled receptors, are necessarily involved in the mechanism of action of antidepressant drugs (3).

Because antidepressant treatment increases monoamine levels and monoamine G protein-coupled receptors are known to undergo homologous down-regulation, the regulation

of the monoamine receptor systems by antidepressants has been extensively studied. Down-regulation of beta adrenergic receptors appears to be a common and very reproducible effect of most tricyclic and atypical antidepressants, as well as electroconvulsive shock. Several studies have reported down-regulation of the alpha-2 adrenergic receptors following chronic antidepressant administration to adult rats.

A major difference between adult and pediatric depression is the response to pharmacotherapy. Tricyclic antidepressants do not appear to be clinically effective in children and adolescents, whereas some SSRIs have been shown to be clinically effective (4). In addition there is concern about the long terms consequences of chronic antidepressant treatment in children (5). In order to develop safer and more effective treatments for pediatric depression, a major thrust of our laboratory has been documenting differences between juvenile and adult rats in relation to the differential response of the pediatric and adult humans to antidepressant drugs. We have focused on five issues: [1] differential maturation of neurotransmitter systems; [2] pharmacokinetics; [3] adrenergic receptor regulation; [4] the alpha-2 adrenergic agonist-mediated hypothermic response; and [5] juvenile animal models of depression.

We have recently reviewed available literature on the development of the mammalian noradrenergic and serotonergic nervous systems, both in terms of neurotransmitter system markers and function (6). In the rat, the norepinephrine system is not fully developed until sexually maturity is reached at about 5 weeks of age, whereas the parameters for the serotonin system reach adults levels two to three weeks earlier. The difference is even more dramatic in the monkey, with some parameters reaching adult levels in two to eight weeks for serotonin, but not until two years for norepinephrine. It is clear from this comparison that the serotonin system reaches maturity much earlier than the norepinephrine system. This temporal difference in the development of neurotransmitter systems may provide at least a partial explanation of the lack of efficacy of antidepressants targeting the noradrenergic system, as compared to the efficacy of those targeting the serotonergic system.

It has been known for many years that pharmacokinetic parameters change as a function of age. For example, after acute i.p. administration desipramine is eliminated from the brain more slowly in postnatal day 21 and 28 rats as compared to adults rats (7). After chronic i.p. administration (for 4-5 days between postnatal day 9 and 28), lower doses of desipramine are needed with juvenile rats to obtain the same brain desipramine concentrations as adults. Thus, the dose of desipramine (and presumable other drugs) needs to be carefully adjusted for juvenile animals in order to have appropriate brain levels of the drug. Whereas desipramine is one of the most selective antidepressants for the norepinephrine transporter as compared to the serotonin transport in both the human and the rat (8), desmethyldesipramine (the major metabolite of desipramine in the rat) has a higher affinity for the rat serotonin transporter (13 nM) than for the rat norepinephrine transporter (153 nM) (9). Treatment of rats with desipramine can result in the concentration of desmethyldesipramine reaching levels similar to that of the parent compound (7). Thus, desmethyldesipramine could contribute significantly to the antidepressant effect of desipramine under certain conditions.

Because the adrenergic nervous system is not fully developed until late adolescence, the mechanisms regulating neurotransmitter receptor density similarly may not yet be mature in young mammals, and thus the response to antidepressants that increase norepinephrine levels may be different in juveniles as compared to adults. Following four days of twice daily injections of desipramine to post-natal day 9-13 (4 and 7 mg/kg/day) and adult (20 mg/kg/day) rats, the beta adrenergic receptor was down-regulated to 50% to 60% of control

in the cortex of both juvenile and adult rats (10). By contrast, in post-natal day 9-13 rats there was a dose-dependent up-regulation of the alpha-2 adrenergic receptor in the prefrontal cortex, whereas there was no change in density in adult. Thus, whereas the regulation of the beta adrenergic receptor appears to the same in the brains of juvenile and adult rats, the regulation of the alpha-2 receptors is strikingly different. This is consistent with the evidence that the alpha-2 receptor plays a major role in depression and in the action of antidepressant drugs (11, 12). The difference in the alpha-2 adrenergic receptor regulation following desipramine treatment suggests that the lack of efficacy of tricyclic antidepressants in treating pediatric depression may be related to immature regulatory mechanisms for this receptor.

To assess a functional response to alpha-2 agonists, we used brimonidine-induced hypothermia (13). Brimonidine, alone, lowered rectal temperature to a greater extent in juvenile than in adult rats. Acute desipramine administration lowered rectal temperature in the absence of brimonidine in adult but not in juvenile rats. The adult rats developed tolerance to this hypothermic effect after 4 days of desipramine treatment. Repeated desipramine treatment of adult rats also resulted in an enhancement in the brimonidine-induced hypothermic effect 24 h after the last dose, a time when above 90% of desipramine and its metabolite, desmethyldesipramine, had cleared the brain (13). In juvenile rats repeated injections of desipramine had no effect on the alpha-2 agonist-induced hypothermia (13). These results suggest that in the juvenile animal at some point in the signal transduction system pathway starting with the inhibition of the norepinephrine transporter and ending with the hypothermic response, there are one or more immature components, which might be related to the lack of efficacy of TCAs in juveniles.

Of the available animal models of human depression, the forced-swim test and the learned helplessness paradigm are the best replicated and accepted. The forced-swim test, also known as behavioral despair, consists of forcing rodents to swim in a confined space on two occasions and is widely used to predict antidepressant efficacy (14, 15). In the learned helplessness model, following inescapable shock stress, rats fail to escape the shock when given the opportunity to do so (16). Although the proportion of animals which demonstrate learned helplessness after inescapable stress varies depending on factors such as strain, duration of inescapable stress, and the escape test used, this model replicates an important feature of human depression in that similar stress results in some individuals developing depression whereas others do not.

In forced-swim test using 21-day old animals, the SSRIs fluoxetine (at 10 mg/kg) and escitalopram (at 10 and 20 mg/kg) increased swimming, but not climbing, behavior just as seen in the adult (17). In stark contrast, the TCA imipramine (at 10 mg/kg) and desipramine (at 10 and 20 mg/kg) did not increase climbing behavior, as occurs in adult animals. In fact, these two antidepressant drugs had no significant effect on any of the three behaviors (17). These data suggest that the juvenile forced-swim test in the 21-day old rat will be useful in evaluating potential new treatments of childhood and adolescent depression.

Chronic treatment of 21-day old rats in the learned helplessness model with escitalopram (10 mg/kg) showed a marked decrease in the mean escape latency times compared to saline-injected control animals (18). This decrease in mean escape latency for escitalopram-treated animals is indicative of a prevention of learned helplessness, which was present in the saline controls. In contrast to escitalopram treatment, 21-day old animals that received chronic treatment with 10 or 15 mg/kg of desipramine did not show decreased latency times when compared to saline controls (18). These data suggest that using 21-day old juvenile rats in the leamed helplessness paradigm is a valid model of antidepressant

action in pediatric depression. This newly-adapted juvenile model of depression should be useful in developing new treatments for childhood and adolescent depression.

Basic research directed toward the understanding of the differences between the responses of juvenile and adult animals to drug administration must take into account several factors. First are the developmental aspects of the systems under study, particularly in terms of the signal transduction pathway (pharmacodynamics). Second are the difference in the pharmacokinetics, including differences in dose administered and the half-life of the agent. The antidepressant-like effects of desipramine, but not citalopram, are very different in juvenile as compared to adult rats. These differences mirror those seem in humans, and appear to result, at least in part, from the differences in the rate of maturation of the serotonin and norepinephrine nervous systems.

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Session VI: Pharmacology of Emotional Disorders

Neuroscience, Bhuddism, and Emotional Disorders

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Abstract

The discussion in this session will be the overlook on the concepts of how drug molecules act on brain molecules and how these events are implicated in altering emotional disorders. The mind in behavioral science interpretation and the mind from Buddhism perspective are reviewed. Examples of neuroscience studies to pin-point brain areas and neurotransmitters involved in mental function will be briefly summarized. Buddhism practices, the approaches taught by Lord Buddha to alleviate unhappiness (took), are believed to be the best treatment for our troubled mind. So, how about the drug treatments in emotional disorders?

ยากับการออกฤทธิ์ต่อสมอง และรัก โลภ โกรธ หลง

ส่วนแรกในบทความที่นำเสนอนี้ มีวัตถุประสงค์เพื่อให้ผู้อ่านเห็นภาพรวมทั้งแนวกว้างและเชิงลึกในบางจุด เกี่ยวกับการออกฤทธิ์ของยาต่อสมอง ในมุมมองที่ไม่ค่อยมีการนำเสนอในวิชาเภสัชวิทยา อาจจะไม่ชัดเจนเท่าที่ควรเพราะยังมีประเด็นที่ไม่เข้าใจกันอยู่มาก แต่หวังว่าท่านที่สนใจจะได้ประโยชน์บ้าง และอาจนำไปหาทางศึกษาเพิ่มเติมได้ต่อไป ในส่วนหลังของบทความ จะนำเสนอข้อมูลการศึกษาเรื่องจิต อารมณ์ เปรียบเทียบทางพฤติกรรมศาสตร์ ทางประสาทวิทยาศาสตร์ และทางพุทธศาสนา ซึ่งก็อาจไม่ชัดเจนอีกเนื่องจากผู้เขียนก็รู้เพียงผิวเผิน แต่คาดว่าน่าจะมีข้อมูลใหม่สำหรับผู้อ่านที่สนใจ และเป็นประโยชน์สำหรับผู้ที่จะศึกษาในเรื่องเหล่านี้

ยา เป็นโมเลกุลหนึ่ง (หรือหลายโมเลกุลรวมกัน) เรารู้สูตรโครงสร้างทางเคมีของยาส่วนมากอย่างดี ความสัมพันธ์ระหว่างสูตรโครงสร้างและการออกฤทธิ์ของยาเป็นเรื่องที่มีข้อมูลชัดเจน เรียกว่ามี Structure-activity relationship (SAR) ถ้ายานั้นมีการจัดโมเลกุลในทิศทางที่ต่างกันได้ (มี stereoisomers) ก็จะมีลักษณะเฉพาะของการจัดทิศทางโมเลกุล (stereospecific) ในการออกฤทธิ์ด้วย เป็นข้อมูลพื้นฐานที่นักเภสัชวิทยาได้รับรู้ทุกคน แต่มีนักเภสัชวิทยาจำนวนน้อยที่สนใจลึกลงไปกว่านั้นว่าคุณสมบัติอะไรในลักษณะโมเลกุลนั้นๆ ที่ทำให้ยามีฤทธิ์หรือไม่มีฤทธิ์ เป็น agonist, partial agonist, antagonist อะไรเป็นปัจจัยตัดสินลักษณะเหล่านี้ กลุ่มที่สนใจด้าน quantum chemisty จะศึกษาข้อมูลลึกลงไปถึงลักษณะการกระจายของพลังงานในโมเลกุลของยาว่าเป็นเช่นไร นำไปสู่ข้อมูลที่เป็น quantal structure-activity relationship (QSAR) และนำไปประยุกต์ใช้ในการหารูปแบบโมเลกุลที่มีลักษณะอย่างใดอย่างหนึ่ง หรือออกฤทธิ์เฉพาะในแบบที่ต้องการ มีการนำข้อมูลและเทคนิคทางคอมพิวเตอร์มาใช้

ช่วยให้สามารถศึกษาข้อมูลจำนวนมากๆ ได้สะดวกขึ้น เร็วขึ้น นำไปสู่การพัฒนาโมเลกุลยาใหม่ๆ ออกมาได้มากในเวลาอันรวดเร็ว (แต่นี่ก็ไม่ได้หมายความว่าการรักษาโรคจะดีขึ้นเสมอไป) ความสนใจในเชิงลึกเช่นนี้ในกลุ่มนักเภสัชวิทยาในบ้านเรายังมีอยู่จำนวนไม่มาก แต่มีนักเคมีและชีวเคมีหลายคนที่มีข้อมูลความรู้ในเรื่องนี้ดี

เมื่อกล่าวถึง quantum chemisty หรือ quantum physics เราได้เรียนกันในปีต้นๆ ของมหาวิทยาลัย แต่ไม่ค่อยได้นำมาใช้ อันที่จริงเป็นวิชาที่ทำให้มองเห็นภาพการเกิดปฏิกิริยาระหว่างโมเลกุลต่างๆ ได้ดีขึ้น วิชาควอนทั่มที่ว่านี้ จะมองว่าอิเลคตรอนไม่อยู่กับที่ แต่วิ่งรอบอะตอมและรอบโมเลกุล ต้องคำนวณโดยสมการหาความเป็นไปได้ที่จะพบว่าอิเลคตรอน (หรือ particle ใดๆ) อยู่ในตำแหน่งใดบ้าง ทำให้คำนวณเป็นรูปร่างอะตอมหรือโมเลกุลได้ แทนที่ความเชื่อเดิมว่าอิเลคตรอนอยู่กับที่ การวิ่งของอิเลคตรอนมีพลังงานเกี่ยวข้องอยู่หลายแบบรวมทั้งการดึงดูดกับโปรตอน แรงเหวี่ยงหนีแรงโน้มถ่วง การเกิดพลังงานแม่เหล็กไฟฟ้า เป็นต้น พลังงานของโมเลกุลเหล่านี้สามารถอธิบายคุณสมบัติทั้งหมดของยาตามที่กล่าวมาแล้วหรือไม่ ประเด็นนี้เชื่อว่ายังไม่มีใครตอบได้

ความเข้าใจเรื่องส่วนประกอบและโครงสร้างของอะตอมและโมเลกุลจากวิชาควอนทั่มนำไปสู่ความรู้ ความเข้าใจทางฟิสิกส์ เคมี และชีววิทยามากมาย แต่การทำความเข้าใจโครงสร้างอะตอมก็ยังไม่จบ เล็กกว่าโปรตอนและอิเลคตรอนยังมีการค้นพบ particle ที่เล็กกว่านั้นลงไปอีกเรื่อยๆ (เช่น quark) และในที่สุดอะตอมจะประกอบด้วยอะไร การคิดค้นของไอนสไตน์เรื่องการเปลี่ยนสถานภาพจากสสารไปเป็นพลังงานได้ ทำให้มีผู้คิดต่อไปว่าในที่สุดอะตอมก็จะเป็นเพียงคลื่นพลังงานก็ได้ (ทฤษฎี string เชื่อว่าทุกสิ่งทุกอย่าง รวมทั้งจักรวาพ มีลักษณะเป็นคลื่น) ไม่มี particle อะไรเป็นชิ้นเป็นอันเลย การคิดเช่นนี้จะไปตรงกับคำสอนของพระพุทธเจ้าว่า ทุกสิ่งทุกอย่างเป็นอนิจจัง มีเกิดมีดับอยู่ตลอดเวลา

ขอออกนอกเรื่องไปอีกนิดเกี่ยวกับไอนสไตน์ เข้าบอกว่าเขาเองไม่มีศาสนา แต่ศาสนาที่เขานิยมหรือศรัทธามากที่สุดคือพุทธศาสนา ทฤษฎีที่เขาคิดได้ทุกเรื่องก็เทียบเคียงเข้าได้กับคำสอนในพุทธศาสนา (1) เช่นในเรื่องความเร็วของแสงและเวลา ซึ่งไอนสไตน์พบว่าไม่คงที่ แปรเปลี่ยนไปตามแรงโน้มถ่วงและตำแหน่งในจักรวาล ซึ่งในพุทธศาสนาก็กล่าวถึงเวลาที่ต่างกันในภพภูมิต่างๆ (พรหม เทวดา มนุษย์ สัตว์อสุรกาย)

กลับมาที่การออกฤทธิ์ของยาที่ตัวรับ (receptor) สมัยก่อนที่ไม่มีข้อมูล นักเภสัชวิทยาก็สมมุติตัวรับเป็นกล่องดำไม่อธิบายอะไร บอกว่ายาจับกับตัวรับแล้วเกิดการตอบสนอง ต่อมามีทฤษฎี occupancy บอกว่าปริมาณการจับมากมีการตอบสนองมาก ต่อมามียาบางตัวจับกับตัวรับได้ดีแต่ไม่เกิดการตอบสนองโดยตรง (antagonist) ก็หาคำอธิบายเพิ่มมาอีกว่า agonist มี intrinsic activity หรือกำลังภายใน = 1 ในขณะที่ antagonist มีกำลังภายใน = 0 กำลังภายในที่ว่านี้คืออะไร อธิบายได้ด้วยอะไร ลักษณะโมเลกุลตามหลัก ควอนทั่ม อธิบายได้ทั้งหมดไหม เชื่อว่ายังไม่มีคำตอบ หวังว่าจะมีนักเภสัชวิทยารุ่นน้องหาคำตอบในเรื่องนี้ได้ดีขึ้นในอนาคต ถ้าโมเลกุลของยาเป็นเพียงคลื่นพลังงานและโมเลกุลของตัวรับในสมองก็เป็นคลื่นพลังงาน การตอบสนองต่างๆ ก็เป็นคลื่นพลังงาน การรับรู้ (เวทนา) การจดจำ (สัญญา) การปรุงแต่งของ

จิตให้เกิด รัก โลภ โกรธ หลง (สังขาร) ก็เป็นคลื่นพลังงาน มีเกิดมีดับไม่มีอะไรเป็นตัวตนของจริง ถ้าอย่างนี้นักเภสัชวิทยาจะใช้เครื่องมือใดมาวัดพลังงานที่ว่านี้ได้ไหม

สมองกับจิต

แนวความคิดและปรัชญาที่เกี่ยวข้องกับการศึกษาเรื่องจิตมีหลากหลายแนวทาง(2) กลุ่มที่สนใจด้านพฤติกรรมจะมองเพียงว่าสิ่งกระตุ้นอย่างหนึ่งทำให้เกิดการตอบสนองเป็นพฤติกรรมอะไร พวกจิตนิยม (idealism) แยกว่าจิตกับสสารไม่เกี่ยวกัน เมื่อกายตายไป จิตหรือวิญญาณก็จะล่องลอยไป พวกสสารนิยม (materialism) เชื่อว่าจิตหรือวิญญาณเป็นผลของสสาร เช่นเป็นการทำงานของสมอง สสารเป็นสิ่งที่มีอยู่แท้จริง จิตและพลังงานต่างๆ เป็นผลงานของสสาร และพวกที่ชอบย่อ (reductionist) ก็จะคิดลงไปถึงหน่วยเล็กๆ ว่าเหตุการณ์ทั้งหมดของชีวิตหรือจิตเกิดจากเหตุการณ์ระดับโมเลกุลหรือปรมาณู ส่วนพวกที่มองแบบคอมพิวเตอร์เห็นว่าสมองก็เหมือนเป็น hardware ของคอมพิวเตอร์ ส่วนจิตเหมือนเป็น Software ซึ่งขึ้นกับการจัดลำดับเชื่อมต่อและบทบาทของส่วนต่างๆ

ข้อมูลทางเภสัชวิทยาที่ใช้อธิบายเรื่องจิต คือการใช้ข้อมูลที่ยาหรือสารเคมีไปมีผลเกิดการเปลี่ยนแปลงของจิต การออกฤทธิ์ของยาทุกชนิดเป็นการเปลี่ยนอัตราของขบวนการที่มีอยู่แล้ว ไม่ใช่เป็นการสร้างขบวนการใหม่ขึ้นมา ยาไปเปลี่ยนแปลงขบวนการในสมองและจิตที่มีอยู่แล้ว มิใช่เป็นเหตุให้เกิดจิต

ตัวอย่างขบวนการในสมองโดยเฉพาะอย่างยิ่งระดับการส่งสัญญาณประสาทต่างๆ นั้น มีข้อมูลมากมายอยู่แล้วว่ามียาไปเปลี่ยนแปลงขั้นตอนเหล่านี้อย่างไรและนำไปสู่การเปลี่ยนแปลงภาวะของจิตอย่างไร แม้แต่ยาโบราณ เช่น ฝิ่น กัญชา มีผลต่อการรับรู้ อารมณ์ ความคิด ก็ทราบกันละเอียดมากขึ้นแล้วว่าในสมองมีระบบส่งสัญญาณประสาทที่รับการออกฤทธิ์ของสารพวกนี้อย่างเฉพาะเจาะจง ยาบ้า ซึ่งมีผลทำให้จิตของผู้เสพมีภาวะบ้าขึ้นมา เราก็พอจะอธิบายได้ว่า มันไปออกฤทธิ์ต่อระบบใดบ้าง

ความพยายามที่จะเชื่อมโยงระบบส่งสัญญาณประสาทชนิดหนึ่งกับภาวะทางจิดแบบใดแบบหนึ่งก็มีข้อมูลอยู่มาก ถึงแม้ว่าจะไม่สามารถเชื่อมต่อกันชัดเจนก็ตาม เช่น ข้อมูลที่สนับสนุนว่าการส่งสัญญาณประสาทโดปามีนที่ limbic system เกี่ยวข้องกับการเกิดอาการโรคจิตเภท noradrenaline และ serotonin เกี่ยวข้องกับอาการซึมเศร้า GABA เกี่ยวข้องกับอาการวิตกกังวล enkephalin เกี่ยวข้องกับอารมณ์และการรับรู้ความเจ็บปวด glutamate receptor (NMDA) ที่ hippocampus เกี่ยวข้องกับการเรียนรู้และการจำเป็นต้น

ตัวอย่างการศึกษาลึกลงไปที่ hippocampus ของหนูที่ฝึกให้เรียนรู้ตามขั้นตอนต่างๆ แล้ววัดการตอบสนองที่เซลล์ประสาทกลุ่มต่างๆ พบว่ามีเซลล์ประสาทเป็นหย่อมๆ ที่ตอบสนองต่อวิธีการเรียนรู้ด้วยวิธีการต่างๆ ไม่เหมือนกัน กลุ่มที่ศึกษานี้ถึงกับเสนอว่า น่าจะสามารถหาข้อมูลต่อไปได้ว่าเซลล์กลุ่มใดและสารส่งสัญญาณประสาทใดตอบสนองต่อการเรียนรู้ชนิดใด(3)

ข้อมูลที่กล่าวถึงแล้วว่าสนับสนุนว่าสารส่งสัญญาณประสาทใดเกี่ยวข้องกับอาการทางจิตแบบใดนั้น เป็นข้อมูลเชิงสรุปให้ง่าย (simplify) เพื่อให้เข้าใจง่ายขึ้นเท่านั้น แต่ในรายละเอียดยังมีข้อถกเถียงได้ทุกกรณี และข้อที่จะต้องนำมาพิจารณาด้วยเสมอก็คือว่า ไม่มีการทำหน้าที่ของสมองในเรื่องใดที่เกิดจากเซลล์หรือสารส่งสัญญาณประสาทกลุ่มเดียว ทุกเรื่องเป็นการมีปฏิกิริยาต่อกันของเซลล์ประสาทหลายกลุ่ม และใช้กายวิภาคของสมองหลายส่วน ผลสุดท้ายที่ทำให้เกิดการตอบสนองในเรื่องที่วัดอยู่ เป็นผลรวมจากการตัดสินการทำงานร่วมกัน พยาธิสภาพที่เกิดขึ้นก็มักจะเป็นการเสียสมดุลของการทำหน้าที่หลายๆ ส่วน และเชื่อว่ายาที่รักษาอาการทางจิตได้ เป็นการไปปรับสภาพสมดุลดังกล่าวให้ดีขึ้น

จิตในพุทธศาสนา

ได้กล่าวถึงการศึกษาจิตจากนักปรัชญาและนักวิทยาศาสตร์ระบบประสาทไปแล้ว แต่ไม่มีข้อมูลอธิบายให้ชัดเจนว่าจิตมีที่มาอย่างไร ในทางพุทธศาสนาแบ่งขันธ์ 5 เป็นรูปธรรม คือรูปหรือกาย กับนามธรรม 4 อย่าง คือ เวทนา (การรับรู้) สัญญา (การจดจำ) สังขาร (การปรุงแต่งให้ชอบหรือไม่ชอบ) และวิญญาณ (ความรู้แจ้ง-รู้ตัว) ส่วนของนามธรรมทั้ง 4 ขั้นตอนนี้เป็นส่วนที่เป็นการทำงานของจิต (1,4,5)

ขั้นตอนการรับรู้ของจิตเกิดผ่านอายะตนะภายนอก คือ รูป เสียง กลิ่น รส สัมผัส อารมณ์ ไปสัมผัสอายะตนะภายใน คือ ตา หู จมูก ลิ้น ผิวกาย ใจ เกิดเจตสิกได้ 57 รวมกันเป็นจิต 121 แบบ มีอะไรบ้างข้อนี้พระท่านว่าไม่ต้องไปใส่ใจเพราะรายละเอียดพวกนี้รู้แล้วก็ไม่ใช่ทางดับทุกข์

เมื่อพิจารณาข้อมูลทางพุทธศาสนาแล้ว อาจจะแยกได้ว่ายาจะไปมีผลต่อการทำงานของจิตที่ระดับใดบ้าง คือ ระดับการรับรู้รูป รส กลิ่น เสียง สัมผัส อาจจะเรียกว่ายาที่ไปลดเวทนา ไปลดการจดจำอาจเรียกว่าไปลดสัญญา ส่วนพวก tranquillizers ไปลดความชอบ พึงพอใจ ติดใจ วิตกกังวล ซึมเศร้า จะเรียกไปลดสังขารได้ไหม และยาที่ไปกดความรู้ว่าอะไรจริงไม่จริง หรือทำให้ความคิดความรู้ตัวผิดไป หลงผิดประสาทหลอนไป อาจเป็นการไปกดวิญญาณไปเลย ศัพท์พวกนี้ยังไม่เคยเห็นใครนำเสนอเลย อาจเห็นว่าเพี้ยนหรือข้ำๆ แต่ลองพิจารณาดู อาจเกิดแนวทางศึกษาใหม่ๆ ได้ (<u>หมายเหตุ</u> วิญญาณในขันธ์ 5 คือวิญญาณรู้ มีผู้รู้เทียบกับคำภาษาอังกฤษว่า น่าจะเป็น consciousness ไม่ใช่วิญญาณที่ล่องลอยออกจากร่างตามความเข้าใจ หรือความเชื่อของคนทั่วไปและในทางพุทธก็ไม่ได้สอนว่ามีวิญญาณหรือจิตล่องลอยออกจากร่างไปเข้าทรงบอกโน่นบอกนี่ได้ หรือเป็นผีไปหลอกชาวบ้านก็ไม่มี แต่บอกว่าจิตเมื่อออกจากร่างก็จะไปปฏิสนธิในภพใหม่ตามสภาวะจิตและวิบากกรรมที่มี แต่ข้อมูลเหล่านี้พระพุทธเจ้าเห็นว่าเป็นเรื่องไม่ควรคิดไม่ควรถาม เป็นอจินไตย ถ้าไม่มีประสบการณ์เองจะเข้าใจไม่ได้และไม่ใช่ทางดับทุกข์)

ในทางพุทธศาสนาการจะดับเวทนา ลดสัญญา สังขาร การปรุงแต่งได้นั้น จะต้องอาศัยวิธีการทางจิตเท่านั้น คืออาศัยสติ (ความระลึกได้อยู่เสมอว่ากำลังทำอะไร คิดอะไร) สมาธิ (นิ่งอยู่กับสิ่งที่ทำ) และปัญญา พิจารณาเหตุและผล เช่น ในเรื่องขันธ์ 5 มรรค 8 ไตรลักษณ์อนิจจัง ทุกขัง อนัตตา และทางสายเอก ที่จะทำได้คือ วิปัสสนาโดยสติปัฏฐาน 4 (1,4,5) จะเห็นว่าแนวทางปฏิบัติในการทำงาน ใช้ชีวิต หรือดับทุกข์จะเริ่มด้วยสติมาก่อนเสมอ พอสติหลุดความคิดต่างๆ ก็จะนำไปในทางทุกข์ หรือทุกข์เพราะคิดผิด (6)

วิธีการที่จะลดความคิด การปรุงแต่ อุปาทาน การยึดมั่น อย่างนั้นถูก อย่างนี้ผิด อยากเห็นเป็ดเป็นไก่ อยากให้คนอื่นทำเหมือนที่ตัวเองชอบ วิธีทางพุทธจะเห็นสติมาก่อนให้รู้เท่าทันว่ากำลังคิดอะไร แล้วใช้ปัญญาพิจารณาว่าอะไรเป็นเหตุเป็นผล จากคิดผิดมาเป็นคิดถูก จะสุขหรือทุกข์ก็อยู่ที่วิธีคิดนี่แหละ (6-9)

ถ้าจะถามลึกลงไปอีกก็น่าสนใจว่าอะไรทำให้คนคิดไม่เหมือนกัน ทางพุทธจะกล่าวถึงวิบากกรรมที่ติดตามไปหลายภพหลายชาติ บวกกับกรรมในชาตินี้ นักวิจัยประสาทวิทยาจะหาคำตอบให้ชัดเจนขึ้นได้หรือไม่ว่าคนที่คิดไม่เหมือนกันเพราะมีกลุ่มเซลล์ประสาทในจุดใดไม่เหมือนกัน synapses ไม่เท่ากัน หรือการเกิดสมดุลระหว่างสารส่งสัญญาณประสาทตัวใดไม่เหมือนกัน ตัวรับและลำดับขั้นตอนการตอบสนอง (signal transduction)ใดที่ไม่เหมือนกัน เป็นต้น แม้ในทางพุทธจะไม่เห็นว่ามียาตัวใดดับทุกข์ได้ นักเภสัชวิทยาอาจจะไม่เน้นการดับทุกข์ถาวร เพียงแต่ไปเปลี่ยนแปลงทุกข์ คือ อารมณ์ ความคิด การปรุงแต่งให้วิตกกังวล ซึมเศร้า รัก โลภ โกรธ หลง ได้บ้าง ถ้าสามารถเข้าใจขั้นตอนการออกฤทธิ์ของยาที่มีผลต่อความคิดของคนได้ก็จะมีประโยชน์ตามมาอีกมากทีเดียว ยังมีเรื่องที่ท้าทายความสามารถของนักเภสัชวิทยาอีกมากเลย

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Orginal article **P 01**

Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Srinagarind Hospital: A Retrospective Study of Causative Drugs and Clinical Outcome

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Abstract

Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are potentially life-threatening drug-induced cutaneous reactions. The drugs commonly implicated as the cause of these severe reactions vary depending on host factors and the drug prescription pattern in that particular area. The aim of the present study was to explore the epidemiology of SJS and TEN in Srinagarind Hospital, Khon Kaen University, Thailand. The case records of all patients with a clinical diagnosis of SJS or TEN admitted in Srinagarind Hospital during 1995 to 2008 were studied in detail regarding the drugs implicated as the causes, management and clinical outcomes. A total of 132 cases of SJS and 29 cases of TEN were identified. The mean age of the patients was 36.6±20.4 years. About 77% of cases were possibly caused by drugs. The culprit drugs were included anticonvulsants (38.71%), antibiotics (38.71%), anti-gout (8.06%), anti-tuberculosis (4.84%) and non-steroidal anti-inflammatory drugs (4.84%). In addition, the common drug implicated as the cause of SJS/TEN was carbamazepine (24.19%), sulfonamides (20.97%) and allopurinol (8.06%). The time from the first exposure to the culprit drugs to the onset of SJS and TEN was 9.56 ± 9.47 days. Length of hospitalizaion was 13.19 ± 12.79 days. The mortality rate was 1.86% (2 cases of SJS and 1 case of TEN). These information will help physicians and pharmacists to be aware of the culprit drugs that caused SJS and TEN in a Thai population.

Keywords: Stevens-Johnson syndrome, Toxic epidermal necrolysis, Epidemiology

Introduction

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are a group of severe life-threatening drug reaction that often linked to drug exposure. Commonly encountered drugs include anticonvulsants, antibiotics and non steroid anti-inflammatory drugs. Epidermal necrosis causes erosions of the mucous membranes, extensive detachment of the epidermis, and severe constitutional symptoms (1,2). SJS is characterized by widespread small blisters, with skin detachment of less than 10% of the total body skin area and transitional SJS-TEN is defined by an epidermal detachment between 10-30% whereas TEN is defined by detachment greater than 30% (3). These severe cutaneous drug reactions are acute, self-limited, with high morbidity, that is potentially life-threatening. Mortailty rates are 5% in SJS, 30-35% in TEN and 10-15% in transitional forms (4). In European countries, the incidence of TEN is estimated at 0.4 to 1.2 cases per million person-years and

of SJS, at 1 to 6 cases per million person-years. Although infrequent, these conditions may cause severely disable or even death in previously healthy people (5,6). The aim of the study was to find the common drugs implicated as the cause of SJS/TEN in the patients admitted in the Srinagarind Hospital and to evaluate the clinical outcome of these severe cutaneous drug reactions.

Methods

Retrospective study was carried out in all case of SJS and TEN that were admitted to Srinagarind Hospital during 1995 to 2008. The case records of all patients including (1) age, sex and race (2) underlying disease and medical history (3) drug intake within two months before the occurrence of SJS or TEN, with dates of introduction and withdrawal of each drug (4) clinical manifestation; and (5) the outcome of treatment; hospital stay and cost of treatment were reviewed. For each drug taken, the timing of use, dose and indication were documented. This study has been approved by the Khon Kaen Ethic Committee for Human Research, Khon Kaen University, Khon Kaen, Thailand.

Results

A total of 132 cases of SJS and 29 cases of TEN were identified. The mean age of patients who suffered from these severe cutaneous reactions was 36.55±20.43 years. Mortality rate for SJS and TEN are 1.86% (3/161). The demographic data of these patients were shown in Table 1.

As shown in Table 2, underlying diseases were detected in about 57.76% of SJS/TEN patients. The most frequent underlying disease among these patients was seizure (14.29%), neuralgia (7.45%) and gouty arthritis (6.83%).

Table 1 Characteristic of Patients with SJS and TEN

	SJS (%)	TEN (%)	Total (%)
Number of patients	132 (81.99)	29 (18.01)	161
Age	34.49 + 20.01	45.90 + 20.00	36.55 + 20.43
Sex			
Male	60 (45.45)	16 (55.17)	76 (47.20)
Female	72 (54.54)	13 (44.83)	85 (52.80)
Number of dead	2 (1.24)	1 (0.62)	3 (1.86)

The culpable drugs were determined in 124 patients (77.02%). The drugs considered responsible for causing SJS or TEN are shown in Table 3. Anticonvulsants and antibiotics were the most common drugs that caused SJS and TEN among these patients and accounted for 38.71% (48/161) of cases. The most common drug implicated as the cause of SJS/TEN was carbamazepine (34.67%) followed by sulfonamide (20.97%) (Table 3). The indications of carbamazepine in these patients included treatments of seizure (35.42%), neuropathic pain (25.00%) and psychotic disorder (14.58%). The other classes of drug implicated for SJS/TEN were allopurinol (8.06%), anti-tuberculosis (4.84%) and nonsteriodal anti-inflammatory agents (4.84%). The mean time from first drug exposure to the onset of SJS or TEN was 9.56 ±9.47 days (n = 41). Clinical manifestation was erythematous macules (91.95%) and mostly involved with mouth and eye for 50.31% and 32.92% respectively. Length of hospitalization was 13.19±12.79 days (n=31). The use of steroid was very common for the treatment of these severe cutaneous reactions (82.00%).

Table 2 Number and Percentage of patients with previous underlying disease

	SJS (%)	TEN (%)	Total (%)
Epilepsy, Seizure	23 (17.42)	0 (0)	23 (14.29)
Neuralgia	10 (7.58)	2 (6.89)	12 (7.45)
Gouty arthritis	8 (6.06)	3 (10.34)	11 (6.83)
Tuberculosis	6 (4.55)	2 (6.89)	8 (4.97)
Infectious	5 (3.79)	2 (6.89)	7 (4.35)
Psychotic disorder	6 (4.55)	1 (3.45)	7 (4.35)
Cerebral infarction	4 (3.03)	1 (3.45)	5 (3.11)
Systemic Lupus Erythematous	4 (3.03)	0 (0)	4 (2.48)
Hypertension	0 (0)	2 (6.89)	2 (1.24)
Diabetes mellitus	1 (0.76)	1 (3.45)	2 (1.24)
Others	8 (6.06)	4 (13.79)	12 (7.45)
No underlying disease	57 (43.18)	11 (37.93)	68 (42.24)
Total	132	29	161

Table 3 Drug implicated as the cause of SJS and TEN

Hospital	SNG		Total
Drug implicated	SJS	TEN	
Anticonvulsants	43 (42.16)	5 (22.73)	48 (38.71)
Carbamazepine	28 (27.45)	2 (9.10)	43 (34.67)
Phenytoin	8 (7.84)	1 (4.55)	16 (12.90)
Phenobarbital	6 (5.88)	1 (4.55)	13 (10.48)
Valproic acid	1 (0.98)	1 (4.55)	2 (1.61)
Antibiotics	39 (38.23)	9 (40.91)	48 (38.71)
Sulfonamides	20 (19.61)	6 (27.27)	26 (20.97)
Penicillin	9 (8.82)	2 (9.10)	11 (8.87)
Amoxicillin	2 (1.96)	0 (0)	2 (1.61)
Others	8 (7.84)	1 (4.55)	9 (7.26)
Allopurinol	7 (6.86)	3 (13.64)	10 (8.06)
Anti-Tuberculosis	4 (3.92)	2 (9.10)	6 (4.84)
Antipyretic-inflammatory	5 (4.90)	1 (4.55)	6 (4.84)
Aspirin	3 (2.94)	0 (0)	4 (3.23)
NSAIDs	2 (1.96)	1 (4.55)	7 (5.65)
Anti-HIV (Nevirapine)	2 (1.96)	0 (0)	2 (1.61)
Miscellaneous	2 (1.96)	2 (9.10)	4 (3.23)
Total	102	22	124

Conclusion

Stevens Johnson syndrome and TEN are generally considered as hypersensitivity reaction to a wide range of etiologic factors, but the majority of cases are believed to be caused by drugs (7). In the present study, 77% of our SJS/TEN cases were clearly drug-induced. The average onset of SJS/TEN was about 9.56±9.47 days. The most common drug implicated as the cause of SJS/TEN in our patients was carbamazepine. Eighty percent of our patients were treated with steroids along with antibiotics and supportive measures. Death in this series occurred 1.86% of patients. The reported mortality rates for SJS and TEN vary from 5% to 70%.

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Original article **P02**

α-Amylase Inhibitory Activity and Anti-hyperglycemic Effect of Cyanidin and Its Derivatives

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Abstract

Cyanidin and its derivatives, the nature anthocyanins, was evaluated their antihyperglycemic activities *in vitro* and *vivo*. Five compounds including cyanidin , cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3,5-diglucoside were determined pancreatic α-amylase inhibition. The results showed that cyanidin-3-rutinoside was the most effective pancreatic α-amylase inhibitor with IC_{50} of 24.45±0.03 μM. When comparing the IC_{50} values, it was found that pancreatic α-amylase inhibitory activity increased in the order of cyanidin-3-rutinoside > cyanidin-3-glucoside > cyanidin > cyanidin-3-galactoside ≅ cyanidin-3,5-diglucoside. However, they were less potent than that of acarbose (IC_{50} = 18.10±0.05 μM μM). Moreover, the rat treated with cyanidin-3-rutinoside at dose of 100 and 300 mg/kg significantly decreased plasma glucose after 60 min starch loading. However, the the group treated with cyanidin-3-rutinoside at dose of 30 mg/kg did not change plasma glucose concentration when compared to control group. These compounds were required to evaluate its toxicity and clinical efficacy for potential application in the prevention and treatment of diabetes mellitus.

Keywords: Cyanidin derivatives, pancreatic α-amylase, acarbose

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolisms, resulting from defects in insulin secretion and/or insulin action at target tissue or both (1-3). One therapeutic approach for treatment of diabetes mellitus is to delay the postprandial hyperglycemia by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase (4-6).

Anthocyanins are a group of naturally occurring phenolic compounds and water-soluble pigments. They are widely distributed in fruits and vegetables of human diets. In addition, anthocyanins showed broad biological activities including antioxidants, intestinal α-glucosidase inhibitory activity (7-8). However there is no literature reported to their α-amylase inhibitory activities.

The objective of the study was to investigate α-amylase inhibitory effect of cyanidin and it derivatives including cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-

galactoside and cyanidin-3,5-diglucoside (Figure 1). Finally, the antihyperglycemic effect of those compounds was performed in normal rats.

Materials and Methods:

1. Inhibitory effect of cyanidin and it derivatives on pancreatic α-amylase :

In the experiment, cyanidin and it derivatives was incubated with starch (1% w/v) in phosphate buffer (pH 6.9). Porcine pancreatic α-amylase (3U/mL) solution were added to the mixtures and incubated at 37 °C for 10 min. Finally, 1% DNS colors reagent solution were added to the mixtures and boiled at 100 °C for 10 min, The reaction was stopped by adding 40% sodium potassium tartrate. The absorbance was measured at 540 nm using spectrophotometer. Acarbose was used as positive control in this study.

2. Antihyperglycemic effects of cyanidin derivatives

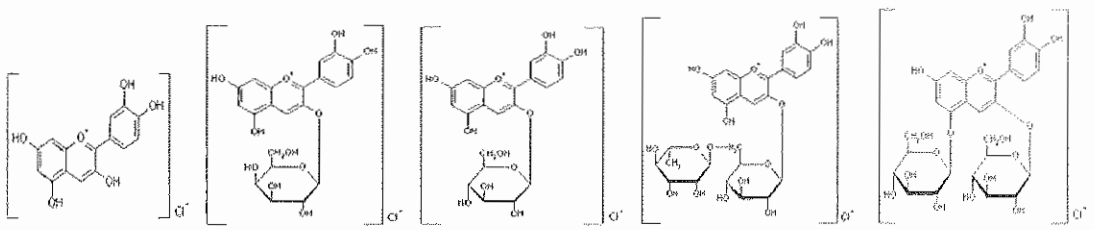
Male rats weighing 250-300 g were allowed free access to an experimental diet for 7 days before starting the experiment. The normal rats were randomly divided into 5 groups of six rats. All groups were co-administrated orally with the starch (5 g/kg) in each rat. Group 1 received distilled water. Group 2, 3, and 4 received cyanidin-3-rutinoside at doses of 30, 100, and 300 mg/kg, respectively. Group 5 received acarbose at doses of 5 mg/kg. Blood samples were collected at 0, 30, 60, 120 and 180 min. The plasma glucose concentration was measured using the glucose oxidase method.

3.Statistical Analysis.

Results were expressed as the mean ± S.E.M. Statistical analysis was calculated by Student's *t*-test, one-way ANOVA and a *post hoc* Least Significance Difference (LSD). $P < 0.05$ was considered to be statistically significant

Results.

The IC_{50} values of cyanidin and its derivatives are on pancreatic α-amylase inhibition are shown in Table 1. Cyanidin and its derivatives significantly inhibited pancreatic α-amylase in dose dependent manner. When comparing the IC_{50} values, it was found that pancreatic α-amylase inhibitory activity increased in the order of cyanidin-3-rutinoside > cyanidin-3-glucoside > cyanidin > cyanidin-3-galactoside ≅ cyanidin-3,5-diglucoside. The results showed that cyanidin-3-rutinoside was the most effective pancreatic α-amylase inhibitor among those of cyanidin derivatives. However, these compounds were less potent than that of acarbose (IC_{50} = 18.10±0.05 μM)



Cyanidin | Cyanidin-3-galactoside | Cyanidin-3-glucoside | Cyanidin-3-rutinoside | Cyanidin-3,5-digluoside

Figure 1. The chemical structure of cyanidin and its derivatives

Table 1. The IC_{50} values of cyanidin and its derivatives on pancreatic α-amylase inhibition

Compounds	IC_{50} (μM)
Cyanidin	381.23±0.02
Cyanidin-3-rutinoside	24.45±0.03
Cyanidin-3-glucoside	302.80±0.05
Cyanidin-3-galactoside	> 1000
Cyanidin-3,5-diglucoside	> 1000
Acarbose	18.10±0.05

Results were expressed as means ± S.E.M; n= 3

From the data mentioned above, cyanidin-3-rutinoside was evaluated its antihyperglycemic activity. The plasma glucose concentrations of normal rats treated with cyanidin-3-rutinoside at 100 and 300 mg/kg (*P<0.05) were significantly decreased in normal rats (Fig. 2) after 60 min starch loading. The area under curve (AUC) was significantly lower than those of normal rats by 5.71 and 8.69 %, respectively (AUC for normal control group =17298 ± 160.52 mg/dL.h.; cyanidin-3-rutinoside 100 mg/kg = 16310 ± 103.59 mg/dL.h; 300 mg/kg = 15795± 136.52 mg/dL.h). There were no differences the AUC of plasma glucose between the groups treated with cyanidin-3-rutinoside (30 mg/kg) and control groups. However, the AUC for the group treated with cyanidin-3-rutinoside was higher than that of acarbose (acarbose = 14901.67±68.69 mg/dL.h).

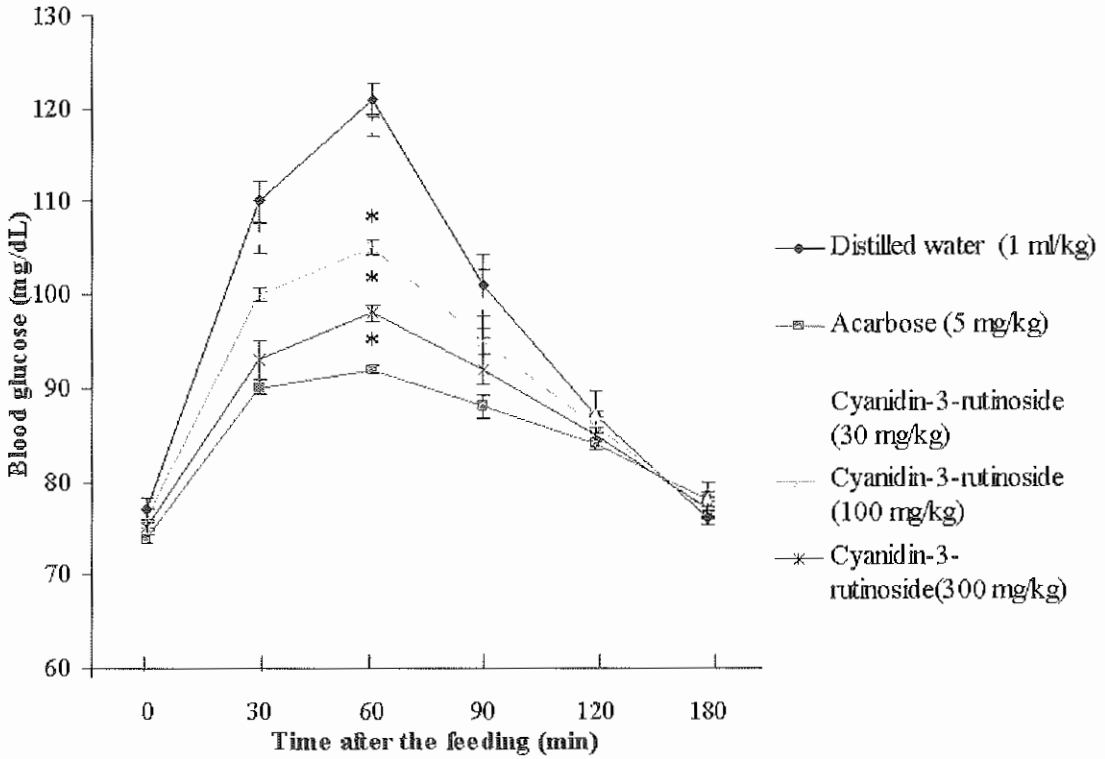


Figure 2. The effect of cyanidin-3-rutinoside on plasma glucose concentration in normal rats Results were expressed as means ± S.E.M; n= 6. *P < 0.05 compared to control group.

Conclusion

The present study shows the potential antihyperglycemic activity of the cyanidin and its derivatives by inhibiting pancreatic α-amylase. Further comprehensive pharmacological investigations of these compounds are required to evaluate its toxicity and clinical efficacy for potential application in the prevention and treatment of diabetes mellitus.

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Original article **P03**

Effect of Ketoconazole on the Pharmacokinetics of Risperidone in Healthy Thai Male Volunteers

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Abstract

Risperidone , a psychotropic agent, is mainly used to treat schizophrenia. The main metabolic pathway is through hydroxylation of risperidone to 9- hydroxyrisperidone by the enzyme CYP 2D6 and to a lesser extent , CYP 3A4. Ketoconazole is an oral antifungal agent of the imidazole class, Ketoconazole is a potent inhibitor of CYP 3A4 activity that can increase plasma concentrations of various drugs , when concomitantly administered during treatment. Therefore, ketoconazole may alter the pharmacokinetics of risperidone when these drugs are coadministered. The objective of this study is to examine the effect of risperidone on the pharmacokinetics of a single oral dose of risperidone in healthy volunteers

In the present study, the pharmacokinetics parameters of risperidone were determined in 10 healthy male volunteers. An open, randomized, two phases crossover design was used in this study. In phases 1, each subject ingested a single dose of 2 mg risperidone alone and in phase 2 , each subject ingested the same dose of risperidone after pretreatment with 200 mg of given orally ketoconazole once daily for 3 days. Plasma concentrations of risperidone at specific times (0,15,30,45 min and1,1.5,2,3,4,5,6,8,12,24,48,72,96 hr) were determined by LC-MS/MS method. The results may be showed that ketoconazole significantly increase the mean C_{max}, T_{max} ,$T_{1/2}$,AUC_{0-48} , $AUC_{0-\alpha}$,Vd and Cl of risperidone when compare with phase1.

In conclusion, the alteration in the risperidone pharmacokinetics parameters may be due to inhibition of CYP 3A4 . Therefore, clinicians should be careful for increasing the dose of risperidone if the patient receives risperidone coadministered with ketoconazole , the dose of risperidone should be adjusted to maximize the therapeutic efficacy and reduce the cost of the therapy. If it possible, ketoconazole should not be coadminitered with risperidone to maximize the therapeutic efficacy of risperidone

Keywords: risperidone, ketoconazole, CYP3A4

Introduction

As ketoconazole is one of azole compounds, a number of side effects are associated with ketoconazole as a result of inhibition of these mammalian enzymes (Venkatakrishnan, 2000). Ketoconazole can inhibit CYP3A4, the major CYP isoform of the liver (Suzuki, 2000). The inhibitions of CYP3A4 results in drug-drug interactions involving ketoconazole can decrease the rate of clearance of many drugs.Since risperidone is metabolized by CYP3A4(Lim YP *et al.*,2009)and this enzyme is also involved in the metabolism of several other drugs (such as alprazolam, atorvastatin, carbamazepine, cyclosporine,

dexamethasone, diltiazem, etc.) so pharmacokinetic interactions due to competitive inhibition of the enzyme may occur. *In vitro* studies showed that those drugs may also be metabolized by other CYP isozymes, including 1A1, 1A2 and 2C9, but they are only weak inhibitors of risperidone metabnolism. Due to the increase in prescribing ketoconazole, the possibility of ketoconazole and risperidone coadministration tends to have a chance to occur in clinical practice, and may lead to cause ketoconazole-risperidone drug interaction. To our knowledge, there are no reports studied on the possible interaction between ketoconazole and risperidone, and the possible role of cytochrome P450 enzymes in the metabolism of risperidone. Therefore, the purpose of this investigation is to study the effect of ketoconazole on the pharmacokinetics of a single oral dose of risperidone in healthy Thai males volunteers, and the present study may be used as a guidance and useful for decision making in case of coadministration of risperidone and ketoconazole in clinical practice.

Materials and methods

1. Chemical Standards and Equipments

Working standard risperidone was purchased from Synfine Research Inc., Canada, Lot. No. A-1208-102,Purity 99.8%. Working standard 9-hydroxyrisperidone was also purchased from Synfine Research Inc., Canada, Lot.No. S-1203-194A1, Purity 98.5%, Carbamazepine (Karindo, Italy) was derived from Regional Medical Sciences Center, Songkhla, Thailand, Lot. No.CB-14/06, Purity 99.92%. Liquid chromatography tandem mass spectrometer consisted of Agilent 1200 series of pump, autosampler and column oven (Agilent Technologies, UK). Ion-trap model of mass spectrometer (LC-MSD trap XCT, Agilent Technologies, UK) was connected to liquid chromatography system as a detector. The column was reverse phase C18, particle size 5 micron diameter 2.1 mm length 150 mm (Alltima HP, Germany). A guard-pak precolumn module was used to obviate rapid column degeneration.

2. Study design

Phase 1: A single oral dose of risperidone (2 mg) alone.

In the morning after an overnight fasting, each subject received a single oral dose of 2 mg risperidone. The drug was administered with a glass of water (240 ml) under supervision. No food was taken at least 2 hours after ingestion of the drug.

A catheter was inserted into a forearm vein for the collection of blood sample, and was maintained patent using 1 ml of a dilute heparin solution (100unit/ml) after each sample. Venous blood samples (5 ml) were collected in heparinized tubes before drug administration and at the time interval: pre-dose, 0.15, 0.5, .075, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 hours after drug administration. Within 30 minutes after collection, all blood samples were centrifuged at 3,000 rpm for 10 minutes under the temperature 4 °C. Collected plasma sample were stored at -20 °C until analysis.

Phase 2: Ketoconazole and a single oral dose of risperidone.

After 2 weeks of being free from the drug, the subjects received ketoconazole capsules at an oral dose of 200 mg (1 capsule) once daily after breakfast for 2 days prior to single oral dose of risperidone administration. In day 3 (after ketoconazole pretreatment for 2 days), after an overnight fasting, all subjects took 2 mg risperidone orally after ketoconazole administration for ½ hour. Venous blood samples were collected at the time interval before and after risperidone administration as previously done in phase 1.

Results

Pharmacokinetics of risperidone alone in the single dose.

After a single oral dose of 2 mg of risperidone alone in ten subjects, the mean C_{max} of risperidone of 15 ±5.67 ng/ml was reached at 0.9 ± 0.13 hours. The mean values of risperidone for AUC_{0-48} was 3.1568 ± 2.201 ng/l.hr . The mean values of risperidone for $AUC_{0-\infty}$, $t_{1/2}$, Cl/f , and Vd/f were 95.75 ± 57.66 ng/l.hr , 5.14 ± 4.85 hr, 0.0234 ± 0.026 l/hr/kg and 0.1447 ± 0.0647 l/kg respectively.

The active metabolize of risperidone was 9-hydroxyrisperidone The mean values of 9-hydroxyrisperidone for C_{max}, T_{max} AUC_{0-96}, $AUC_{0-\infty}$, $t_{1/2}$, Cl/f , and Vd/f were 11.956 ± 6.477 ng/ml, 7.15 ± 4.096 hours, 18.296 ± 5.691 ng/l.hr , 306.6 ± 145.8 ng/l.hr , 19.364 ± 5.323 hours , 0.0086 ± 0.0056 l/hr/kg , 0.2381 ± 0.1583 l/kg respectively.

Pharmacokinetics of risperidone in subjects after pretreatment with ketoconazole compared with risperidone alone.

The mean plasma concentration-time profile of risperidone was show in figure 10. The mean values of C_{max}, T_{max}, AUC_{0-48} , $AUC_{0-\infty}$, $t_{1/2}$, Cl/f , and Vd/f were 20.188 ± 4.69 ng/ml , 0.975 ± 0.3216 hours , 88.38 ± 57.048 ng/l.hr , 89.678 ± 60.462 ng/l.hr , 3.1568 ± 0.0257 l/hr/kg and 0.1094 ± 0.0508 l/kg respectively (Table 25).The mean plasma concentration-time profile of 9-hydroxyrisperidone was show in figure 11. The mean values of C_{max}, T_{max}, AUC_{0-48} , $AUC_{0-\infty}$, $t_{1/2}$, Cl/f , and Vd/f were 18.296 ± 5.691 ng/ml , 3.675 ± 2.769 hours , 341.76 ± 213.22 ng/l.hr , 348.65 ± 215.8 ng/l.hr , 18.296 ± 5.691 hours, 0.0085 ± 0.006 ng/l/hr and 0.1852 ± 0.1573 l/kg respectively

Conclusion

In the ketoconazole pretreated group, the mean C_{max}, of risperidone and 9-hydroxy risperidone were increased by 20.188% and 53.027% and the T_{max} of risperidone was increased by 8.333% but the T_{max} of 9-hydroxyrisperidone was not significant different from the control group. The T_{max} of 9-hydroxyrisperidone was significantly decreased by 10.278% and the $T_{1/2}$ of risperidone and 9-hydroxyrisperidone were significantly decreased of half life by 38.58% and 24.215%. The AUC_{0-48}, $AUC_{0-\alpha}$ of risperidone were decreased by 4.257% and 6.35% but the AUC_{0-96} , $AUC_{0-\alpha}$ of 9-hydroxy risperidone were increased by 16.375% and 13.698% and the Cl/f of risperidone and 9-hydroxy risperidone were increased by 50.42% and 3.093% but Vd/f of risperidone and 9-hydroxyrisperidone were decreased by 0.238% and 22.268%.The increasing of C_{max} of a single oral dose risperidone after pretreatment with ketoconazole for 3 days may be due to inhibition hepatic CYP3A4, inhibition of gut wall first pass metabolism or inhibition of untestinal CYP3A4 and, saturated first pass metabolism of risperidone but Vd/f of risperidone and 9-hydroxyrisperidone nearly of risperidone and 9-hydroxyrisperidone are decreasing by 24.3% and 22.3% therefore Vd/f is not depended on resulted of other pharmacokinetic parameters of risperidone and 9-hydroxyrisperidone

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Original article **P04**

Antidiabetic Effect of Grape Seed Extract (*Vitis vinifera*) in High Fructose-Fed Rats

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Abstract

Type 2 diabetes is a chronic disease characterized by insulin resistance and/or an inadequate compensatory insulin secretory response. Grape seed extract (GSE) has been reported to possess antidiabetic activity. However, there was no the literature reported to the effect of grape seed extract on plasma glucose in high fructose-fed rats. The present study was performed to investigate the antihyperglycemic effects of grape seed extract in high fructose-fed rats. Phenolic compounds and flavonoids content in grape seed extract was determined by using Folin-Ciocalteu's reagent and $AlCl_3$ assay. Six experimental groups were compared: a control group receiving the control diet, a high-fructose-fed group where 60% of the diet carbohydrate was fructose, a high-fructose-fed group supplemented with 0.5, 1 and 2% GSE, respectively. The sixth group received the high fructose diet and rosiglitazone (4 mg/kg). After 8 weeks of experiment, fasting plasma glucose concentrations were measured and oral glucose tolerance test were performed in each group. Total phenolic compounds and flavonoids content were 2.16±0.01 and 7.39±0.45 mg/g extract respectively. The diabetic rats supplemented with 1% GSE significantly decreased fasting plasma glucose level ($p<0.05$) after 8 weeks of administration. Moreover, the diabetic rats supplemented with GSE at 1 and 2% improved glucose intolerance when compared to the diabetic control group ($p<0.05$). In conclusion, the present results indicated that grape seed extract reduced hyperglycemia in high fructose-induced diabetic rats. Thus, grape seed extract may be useful in the prevention for type 2 diabetes.

Keywords: Grape seed, diabetic rats, high-fructose diet

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and protein metabolism that results from defects in both regulations of insulin secretion and/or insulin action. Diabetes mellitus is associated with a reduced quality of life and increased risk factors for mortality and morbidity. The prevalence of diabetic patients in worldwide has dramatically increased every year because of lifestyle and eating behavior changed by consumption of a high-carbohydrate diet.

Fructose is consumed in significant amounts in both Asian and Western diets. The long term consumption of fructose-enriched diets has been associated with increasing risk factor of obesity and metabolic disorders especially diabetes mellitus. Interestingly, a large amount of fructose or sucrose (>60% of total calories) consumed in human produce a decline in insulin sensitivity in the liver and peripheral tissues (1), leading to development of type 2 diabetes mellitus.

Grape seeds, a rich source of phenolic compounds (2-5), possess a broad spectrum of antioxidative properties that protects various cells from free radicals and oxidative stress. Recently, they have reported to antihyperglycemic activity of GSE in streptozotocin (STZ)-

induced diabetic rats (6-7), however, there was no literature report of the GSE effect in a high fructose-fed rat model. Therefore, the aim of study was to investigate the effects of GSE on plasma glucose concentration in high fructose-fed rats. The oral glucose tolerance tests (OGTTs) was also performed to investigate the ability of glucose utilization in diabetic rats.

Materials and methods

1. Plant material and extraction: Dried grape seed (GS) was supplied from Siam Winery Trading Plus Co., Ltd., Samutsakorn Province, in January 2007. Grape seeds were washed with water at 60 °C for 2 h and then extracted with water at 90 °C for 2 h. The aqueous extract was filtered and freeze dried by lyophilizer and kept at -4 °C until use (8). The dry powder (GSE) was obtained and the yield was about 3%.

2.Estimation of total phenolic compounds by Folin-Ciocalteu colorimetric method: Grape seed extracts were dissolved in distilled water at a concentration of 0.125 g/ml. Aliquots of diluted extracts (200 µl) were added to test tubes and mixed with 1 ml of Folin-Ciocalteu's phenol reagent and 3 ml of 2% sodium carbonate solution and then shaken. The absorbance of the reaction mixtures was measured at 760 nm after 2 h. Gallic acid was used as a standard compound to construct a standard curve (9).

3. Estimation of flavonoids content by aluminum nitrate colorimetric method : Grape seed extracts were dissolved in 80% ethanol at a concentration of 10 mg/ml. Aliquots of diluted extracts (0.5 ml) were added to test tubes and mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 2.5 ml of distilled water. After standing for 30 min at room temperature, the absorbance of the reaction mixtures was measured at 415 nm. Quercetin was used as a standard compound to construct a standard curve (10).

4. Animals and experimental diets: Male Sprague-Dawley rats (150-170 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were housed individually in stainless cages in an air-conditioned room maintained at 25 °C. They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments. All procedures were complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

After five-day acclimatization, the animals were randomly divided into six groups to receive experimental diets. The first group received the normal diet (AIN-93G), whereas the other five groups were fed with fructose-enriched diet. The composition of the control and fructose diets was given in Table 1 (11, 12). The following experimental groups were maintained for a total experimental period of 8 weeks.

Experimental Groups

Group 1 (Control)	: received the normal diet for 8 weeks.
Group 2 (High-fructose)	: received the HF diet for 8 weeks.
Group 3 (0.5%GSE)	: received the HF diet supplemented with 0.5%GSE for 8 weeks.
Group 4 (1%GSE)	: received the HF diet supplemented with 1%GSE for 8 weeks.
Group 5 (2%GSE)	: received the HF diet supplemented with 2%GSE for 8 weeks.
Group 6 (Rosig-treated)	: received the HF diet for 8 weeks; rosiglitazone treatment (0.4 mg/ 100g body wt./day) was started from week 5 of the experimental period.

5. Experimental Protocol

Biochemical Analysis: The rats were fasted overnight and blood samples (0.2 mL) were taken from lateral tail vein after 2, 4 and 6 weeks of the experiments. Glucose was determined by Glucose oxidase test (Sigma Chemical Co., USA). Body weight and food consumption were monitored everyday

Table 1. Composition of the experimental diets (g/kg diet)

Ingredient	Control diet	High-fructose diet
Casein (vitamin free)	200	200
Cornstarch	530	-
Sucrose	100	-
Fructose	-	630
Soybean oil	70	70
Mineral mix	35	35
Vitamin mix	10	10
Fiber	50	50
Methionine	3	3
Choline bitartrate	2.5	2.5

.6. Oral Glucose Tolerance Test (OGTT): One day before the end of the experiment. Each group was fasted overnight and subjected to an oral glucose tolerance test by gavage (2 g glucose/kg body wt.). Blood samples (0.2 mL) were taken from lateral tail vein before and after 15, 30, 60, 90 and 120 min of loading glucose solution. Plasma glucose concentration was determined by glucose oxidase tests. The area under curves (AUCs) for glucose was calculated using the trapezoidal rule.

7. Statistical analysis

Statistical analyses were performed using the SPSS 16.0 package. The results were expressed as means±S.E.M. Data were analyzed by one-way ANOVA and a *post hoc* least significant difference (LSD), $p<0.05$.

Results

Phytochemical analysis: Determination of total phenolic compounds was done by using Folin-Ciocalteu colorimetric method including phenolic acid and flavonoids. Total phenolic contents for GSE were estimated to be 2.16±0.01 mg gallic acid/g of the dried extract. As in the total phenolic compounds, flavonoid content was 7.39±0.45 mg/g of the dried extract.

Effect of GSE on fasting plasma glucose concentrations: Changes in the body weight and the fasting plasma glucose concentrations after administration of GSE for 8 weeks were shown in Table 2. The decreases in body weight in the fructose-induced diabetic rats with GSE at 0.5 and 1% treated were significantly lower compared with diabetic control rats. There were a significant reduction ($p<0.05$) of plasma glucose concentrations in the fructose-induced diabetic rats with GSE at 1% and rosiglitazone when compared to high fructose diet group. The diabetic rats treated with 1%GSE and rosiglitazone tended to plasma glucose concentrations toward near normal level in normal rats.

Table 2 Effects of GSE on body weight and fasting plasma glucose concentrations in diabetic rats

Groups	Body weight (g)		Fasting plasma glucose (mg/dl)	
	Initial	Final	Initial	Final
Normal rats	174±2	388±8	100.0±11.6	128.7±14.2
Diabetic rats				
Control	168±1	402±10	82.8±7.9	165.4±15.7*
GSE 0.5%	158±2	369±12**	93.7±6.3	139.4±5.9
GSE 1.0%	160±2	363±13**	92.2±4.3	132.6±11.4**
GSE 2.0%	165±1	385±4	93.9±5.4	134.5±5.2
Rosiglitazone (4mg/kg)	170±2	423±11	102.8±10.1	130.4±7.1**

Values are expressed as mean±S.E.M. (n=6), *: $p<0.05$ vs normal rats; ** : $p<0.05$ vs diabetic rats

Effect of GSE on oral glucose tolerance test : The plasma glucose concentrations in diabetic rats supplemented with 0.5-2% GSE ($p<0.05$) were significantly decreased after 15 min of glucose loading (Fig1). The reduction of AUC for plasma glucose in the groups supplemented with GSE at 0.5, 1 and 2% were 13%, 23% and 27%, respectively when compared to high fructose-induced diabetic group.

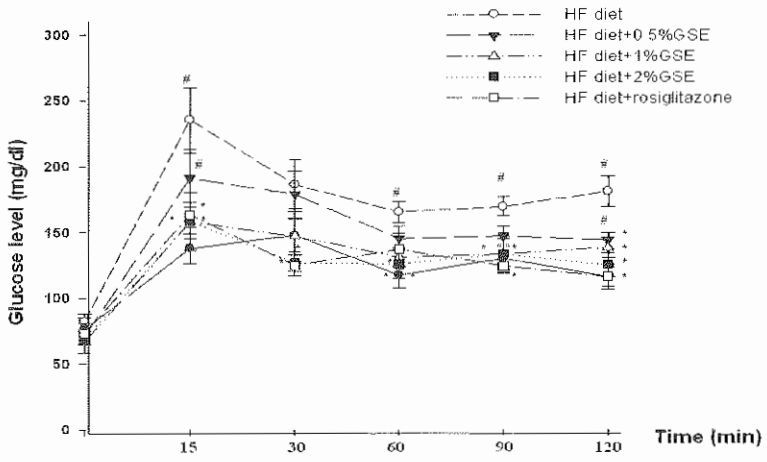

Figure 1 The effects of grape seed extract in diabetic rats by OGTTs. Values are expressed as mean±S.E.M. (n=6). $^{\#}p<0.05$ compared to normal rats; * $p<0.05$ compared to diabetic rats

Conclusion

GSE markedly decreases plasma glucose concentration and also improves glucose intolerance in high fructose-fed rats. Thus, the grape seed extract may be useful in the prevention of type 2 diabetes.

Acknowledgement

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Original article **P05**

NAD(P)H-Quinone Oxidoreductase-1 (NQO1) C609T Genotypes in the Thai population

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Abstract

NAD(P)H-quinone oxidoreductase-1 (NQO1) is a detoxifying/antioxidant enzyme that plays a critical role in cellular defense against reactive oxygen species and toxic quinone derivatives, which in turn confer cytoprotection, inhibition of mutation and carcinogenesis. A polymorphism in human NQO1 at nucleotide position 609 (amino acid codon 187) is associated with diminished NQO1 enzymatic activity, and increased risk of chemically-induced cytoxicity and susceptibility to various forms of cancer. The purpose of this study was to determine the NQO1 polymorphism in the Thai population. We genotyped the NQO1 C609T polymorphism by PCR-RFLP in 189 unrelated healthy Thai subjects. The frequency of NQO1 C609 or wild type allele was 58.6%, where those of C/C, C/T and T/T genotypes were 32%, 53% and 15%, respectively. The frequency of NQO1 C609 allele in Thais was closely related to those observed in the East Asian (Oriental) population. Additionally, Thais exhibited a relatively low frequency of NQO1 C609 allele compared to Caucasian and African-American populations. Since, this is the first report on the NQO1 polymorphism in Thai population, data from this study can be use to further evaluate the impact of NQO1 polymorphism on susceptibility to chemically-induced toxicity and cancer risk.

Keywords: NAD(P)H-quinone oxidoreductase-1 (NQO1), Polymorphism, Thai population

Introduction

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a detoxifying/antioxidant enzyme that plays an important role in protecting cells against chemically induced oxidative stress, cytotoxicity, mutagenicity, and carcinogenicity. NQO1 protects cells from oxidative damage by preventing the generation of reactive oxygen species and reactive electrophiles from certain environmental carcinogens (1). A genetic polymorphism (C→T) at nucleotide position 609 (amino acid codon 187) of the human NQO1 cDNA was show to reduce NQO1 enzyme activity, which may diminish the protection provided by NQO1. Variation in NQO1 enzyme activities has been suggested to influence an individual ability to metabolize carcinogenic agents and thus to be causally linked to cancer risk (2). Over years, a number of studies on the genetic polymorphism of NQO1 gene in many populations have been documented (4-7). However, no study has been reported on the genetic polymorphism of NQO1 gene in the Thai population. In the present investigation, efforts have been made to understand the genetic distribution of NQO1 polymorphism in Thai population.

Materials and methods

1) Study population

The study population consisted of 189 unrelated healthy Thais who were native-born in the northeast region of Thailand (136 men and 53 women; mean age, 48.0±11.4 year). All blood samples were left-over specimens from the project "genetic polymorphism of *N*-acetyltransferases in association with risk of cholangiocarcinoma (HE)".

2) NQO1 C609T by PCR and restriction fragment analysis

The NQO1 C609T polymorphisms was amplified into a 304-bp fragment and subsequent restriction fragment analysis with *Hin*fI as previously described by Seedhouse et al., 2002 (3). All PCR products contain an internal *Hin*fI site and the 609T polymorphism also introduces an additional *Hin*fI site. The wild type or C allele yielded two bands (33-and 271-bp) whereas the mutant or T allele yielded three bands (33-, 120- and 151-bp). The digested products were resolved on 2% agarose gels containing ethidium bromide and analyzed under UV light.

3) Statistical analyses

The observed allele and genotype frequencies of the NQO1 genes were calculated as percentage and 95% confident interval (95% CI). The distribution of genotypes was assessed for significance by χ^2 testing. All calculations were performed with STATA version 8.2.

Results

A representative gel of NQO1 C609T by PCR and restriction fragment analysis is shown in Figure1.

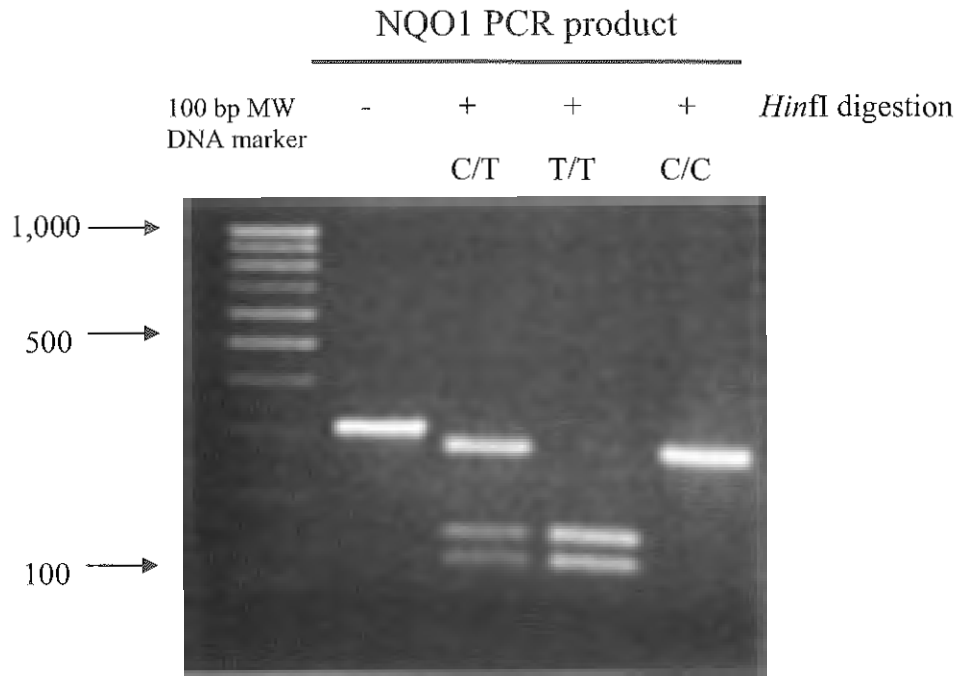

Figure 1. PCR-RFLP analysis of NQO1 C609T on 2% agarose gels.

Using PCR-restriction fragment analysis, NQO1 C609T genotype distribution in the Thai population was determined (Table1). Of the 378 allele tested for the polymorphism, 58.7% (95% CI: 53.8-63.7) were NQO1 C609 or wild type allele. Among 189 subjects, the prevalent of NQO1 C609T carriers (C/T or T/T) was 68%. The observed genotype did not significantly deviate from the expected Hardy-Weinberg equilibrium (χ^2 = 0.89, p>0.05). The frequency of NQO1 C609 allele of Thai resembles that of East Asian (oriental; Korean, Chinese and Japanese) populations in which the frequency of C609 allele was about 50-60% (5-7). This is distinct from those observed in Caucasian (84%) (4) and African-American (81%) (4) populations, where the frequency of NQO1 C609 is relatively high (p<0.0001).

Table 1. Genotype frequencies of NQO1 C609T in healthy Thais.

Genotype for NQO1 C609T	n	Percentage	95% CI
C/C	61	32.3	25.6-38.9
C/T	100	52.9	45.8-60.0
T/T	28	14.8	9.7-19.9
Total	***189***		

Discussion

The NQO1 609T carriers (C/T or T/T) have been suggested to have lowered rate of metabolism (activation/deactivation) of xenobiotics when compared to NQO1 C/C genotype (4-7) which imply that genetic polymorphism can directly affect susceptibility to xenobiotic toxicity, cancer risk and some other diseases. Data from this study provide a background for further epidemiological studies to evaluate the impact of NQO1 C609T polymorphism as a genetic susceptibility gene on disease risk.

Conclusions

The pattern of NQO1 C609T polymorphism in Thais is similar to those in East Asian. This is the first report on NQO1 genotype frequency in the Thai population, data from this study can be use to further evaluate the impact of NQO1 polymorphism on susceptibility to chemically-induced toxicity and cancer risk.

Acknowledgements

This work was supported by in part by the Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University, Khon Kaen, Thailand.

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Original article **P06**

Cytotoxicity Effects of Porcine's Brain Extract Powder

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Abstract

Cytotoxicity study of porcine's brain extract powder (PBEP) using the mouse lung fibroblasts cell line (L-929) following as ISO 10993-5 (1) was investigated. Each six concentrations of PBEP with cultured medium (10, 25, 50, 100, 200 and 500 µg/ml) were added in 24 well plates of L929 cell line seeding ($1x10^5$cells/ml) and incubated in 5 % CO_2 incubator at 37 °C for 24 and 48 h. The cytotoxic effect was then evaluated by qualitative evaluation of cell morphology using inverted microscope. The result of L-929 cell line showed no morphological change in all concentrations of PBEP. Therefore, this study indicated that Procine' s brain extract powder has no cytotoxic effect during 48 h.

Keywords: Porcine's Brain, Cytotoxicity test

Introduction

The previous study of Cerebramin (cerebal cortex of cattle and pig) as cerebral bioregulator effect was associated with the nucleoprotein complex on cerebral cells facilitating restoration of the brain function (2). Thus, the effective form of the animal's brain such as porcine should be the brain extract rather fresh brain. CF1 is cytoplasmic fraction from porcine's brain, which is contained the xenogenic peptide, a cytoplasmic protein. It has been previously reported that substance could penetrate through the target organ elicit the repair mechanism and resulted in the restring the failure function of the cells and finally leading to healthy cells with the protein existed in the cells. Therefore, this raised the possibility that CF1 probably have the potential against Alzeimer's disease (3). Accordingly, in case to further develop porcine's brain extract powder as food supplement, cytotoxicity testing in this study is very necessary.

Materials and methods

1. Cell preparation

A cell line of mouse lung fibroblasts (L-929) was seeded in 25 cm^2 cultured flask using MEM supplemented with 10% horse serum and 1% penicillin-streptomycin and incubated in 5% CO_2 incubator at 37 °C until the cells had been grown as confluent monolayer before starting each cytotoxicity test. The flasks with confluent cells were trypsinized with 0.25% trysin-versene and then seeded in 24 well plates as a density of approximately 1 X 10^5 cells/ml in order to establish 80% confluent monolayer.

2. Reagent Preparation

Porcine's brain extract powder was supported by Pharmaceutical and Natural Products Department, Thailand Institute of Scientific and Technological Research (TISTR), Pathumthani, Thailand.

Six concentrations of porcine's brain extract powder (10, 25, 50, 100, 200 and 500 μg/ml) were dissolved in MEM for cytotoxicity study. The extract solutions were filtered using 0.22 μm syringe filters.

Positive control solution; zinc acetate was dissolved in 0.9 % normal saline and then sterilized with 0.22 μm syringe filters.

3. Cytotoxicity Test

After 24 h of L929 cells were seeded in 24 well plates, the medium (0.5 ml) was replaced with sample extract solution in the range of concentrations comparing with zinc acetate solution and blank in the same plate. This will be done in triplicate and then incubated in 5% CO_2 incubator at 37 °C for 24 and 48 h.. This activity will be conducted to qualitative evaluation.

Qualitative Evaluation: The change in cellular morphology was scored (see grading of scores in Table 1) and compared to the negative and positive control at 24 and 48 h by using an inverted microscope. The score is then used to classify the cytotoxicity effect of sample.

Table 1 Qualitative scores evaluation in the cytotoxicity test. (4)

Score	Reactivity	Change of cellular morphology
0	None	No change
1	Slightly toxic	Slight changes, few cells affected
2	Mildly toxic	Mild changes, many cells round/spindle shaped
3	Moderately toxic	Moderate changes, many cells round/spindle shaped
4	Severely toxic	Severe changes, about all cells show morphological change

Results

The cytotoxic scores of PBEP, positive and negative control are shown in Table 2. After 48 h., the cellular morphology and confluent monolayer growth of L929 cells exhibited normal comparison with control. (Fig.1)

Discussion

This method is useful for verification and determining whether a potential replacement extracted sample is equivalent to the one that is currently being consumed. Therefore, the negative result of PBEP exhibits free of L929 isolated cells damaged and no morphological change. It was indicated that PBEP showed no acute cytotoxic effect. However, the chronic cytotoxic effect of PBEP need further study.

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Table 2 The cytotoxic scores of PBEP samples onto L929 cell line.

No	Sample	Scoring	
		24	48
1	Blank	0	0
2	Negative control	0	0
3	Positive Control	4	4
4	PBEP 10 μg/ml	0	0
5	PBEP 25 μg/ml	0	0
6	PBEP50 μg/ml	0	0
7	PBEP 100 μg/ml	0	0
8	PBEP 200 μg/ml	0	0
9	PBEP 500 μg/ml	0	1

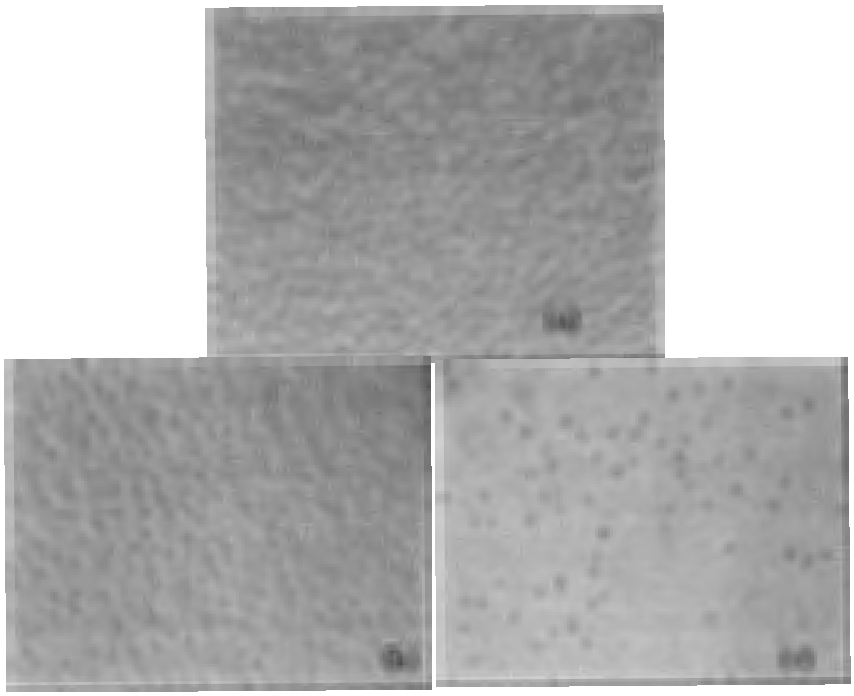

Figure 1: Morphological change of L929 cell 10X, (a) : negative control; (b) : Treated cell; (c) : positive control

Original article **P07**

Lipid Lowering and Vasoprotective Effects of Grape Seed Extract in High Fat Diet-fed Rats

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Abstract

Abnormalities in endothelium-dependent control of vascular tone develop early in the course of atherosclerosis and may result from oxidative modification of low density lipoproteins. This study was aimed to investigate the effects of grape seed extract (GSE) on serum lipoproteins, vascular function and pathological changes of endothelial cell in high fat diet-fed rats. Compared to untreated high fat diet control, GSE significantly reduced serum total cholesterol, LDL-C, HDL-C, and triglyceride levels ($p<0.05$). Endothelial cells and vascular function in GSE-treated groups were improved associated with the increasing of nitric oxide production in blood. This study showed that GSE had beneficial effect on cardiovascular system through its lipid lowering action and endothelial preservation.

Keywords: grape seed extract, hyperlipidemia, vascular function, endothelial cell

Introduction

Hyperlipidemia is one of the major risk factors for developing atherosclerosis, that leading cause of cardiovascular disease. Atherosclerosis induces two significant pathological processes: an ischemia event due to blood flow obstruction and vascular contractile dysfunction, that cause of death among population in developed countries (1). However, the cardiovascular disease mortality rate for France was noted to be lower than observed in other industrialized countries with a similar coronary risk factor profile has been attributed to frequent consumption of red wine and this lead to the term 'French Paradox' (2).

Grapes (*Vitis vinifera*) are one of the most widely consumed fruits in the world and have enormous health benefits. Grape seeds are byproducts of grapes formed during the industrial production of grape juice and wine. They are a potent source of proanthocyanidins which are composed mainly of dimers, trimers and oligomers of monomeric catechins (3). Previous studies demonstrated that grape seed extract increases antioxidant activity in plasma and antioxidants prevent the oxidation of LDL-C and thereby delay atheroma formation (4). *In vitro,* GSE inhibits lipase enzyme and reduce fat absorption to 3T3-L1 adipocytes (5). Other previous studies of GSE demonstrated that GSE has anti-inflammatory and inhibiting effect on platelet aggregation(6). Base on these results, we aimed to investigate further the long term effects of dietary supplementation with grape seed extract on lipoproteins levels. The vascular function and pathological changes of endothelial cell during prolonged hyperlipidemia conditions was also determined.

Materials and methods

Forty male Wistar rats initially weighing 120-150 g were randomly divided into five groups as following: Group 1, normal diet (ND); Group 2, high fat diet (HF); Group 3, HF + fenofibrate 100 mg/kg; Group 4, HF + 0.5% GSE and Group 5, HF + 1% GSE. Each group comprised of 8 rats and all of them were fed for 8 weeks consecutively. Food and water were supplied *ad libitum* for all groups. Blood was obtained prior to dietary treatment and at the time of sacrifice. Plasma was analyzed for the content of total cholesterol, triglyceride and HDL-C using commercial available kits (Human Gesellschaft Biochemical, Germany). Nitric oxide production in blood was assayed by non-enzymatic assay. Low density lipoprotein (LDL) level was derived from the above data using the formula: LDL = Total cholesterol – HDL– (1/5) TG. Aortic ring was isolated and used for the assay of vascular function as described. At the end of selected experiments, a representative rings was fixed and prepared for examination of the pathological changes of vascular cells structure.

Results

After 8 weeks of experiment, the plasma lipid profile in all groups of rat are shown in table 1. Compared to HF group, rats receiving HF diets containing 0.5% and 1% GSE showed significant reductions of plasma total cholesterol, LDL-C, HDL-C and triglyceride level.

The effects of GSE on vascular activity are shown in Fig. 1. NE induced contraction in HF group decreased comparing with ND group while rats treated with GSE demonstrated significantly improved in contractile response induced by NE (figure 1A). The endothelium-dependent relaxation to Ach was significantly impaired in the HF group as compared to ND group, whereas, relaxation to Ach of the GSE treated groups were significantly restored (figure 1B). There was no significant difference in the extent of relaxation to SNP, a direct smooth muscle relaxation and nitric oxide donor among the five groups of rats (figure 1C).

Comparing with the aortae obtained from ND rats (figure 2A), the pathological changes examination revealed the deposit of foam cell and fat in intimal and media layer of those from the HF group. Impaired endothelial cell and migration of vascular smooth muscle cells from media layer into intima were found in this group (figure 2B). The 0.5 and 1% GSE treated groups were slightly found fat deposits. Endothelium cells and vascular smooth muscle cells were still in good condition as showed in figure 2D.

Table 1. Levels of plasma total cholesterol, LDL, HDL and TG in various groups of rats

Groups	Serum lipid parameters (mg/dl)			
	TC	TG	HDL-C	LDL-C
ND (normal diet)	91.29±1.19*	125.24±1.29*	45.21±0.50*	20.97±0.95*
HF (high fat diet)	482.87±8.17	546.42±6.69	121.00±0.75	240.09±13.56
HF+fenofibrate 100 mg/kg	220.92±0.78*	203.81±0.95*	104.10±0.71*	76.05±0.99*
HF+ 0.5% GSE	232.24±1.78*	233.28±0.96*	73.59±0.68*	113.51±2.53*
HF+ 1% GSE	192.24±0.64*	205.02±0.78*	75.91±0.58*	77.37±1.50*

All values are mean ± SEM . (n=8)

* $P < 0.05$ compared to high fat diet group

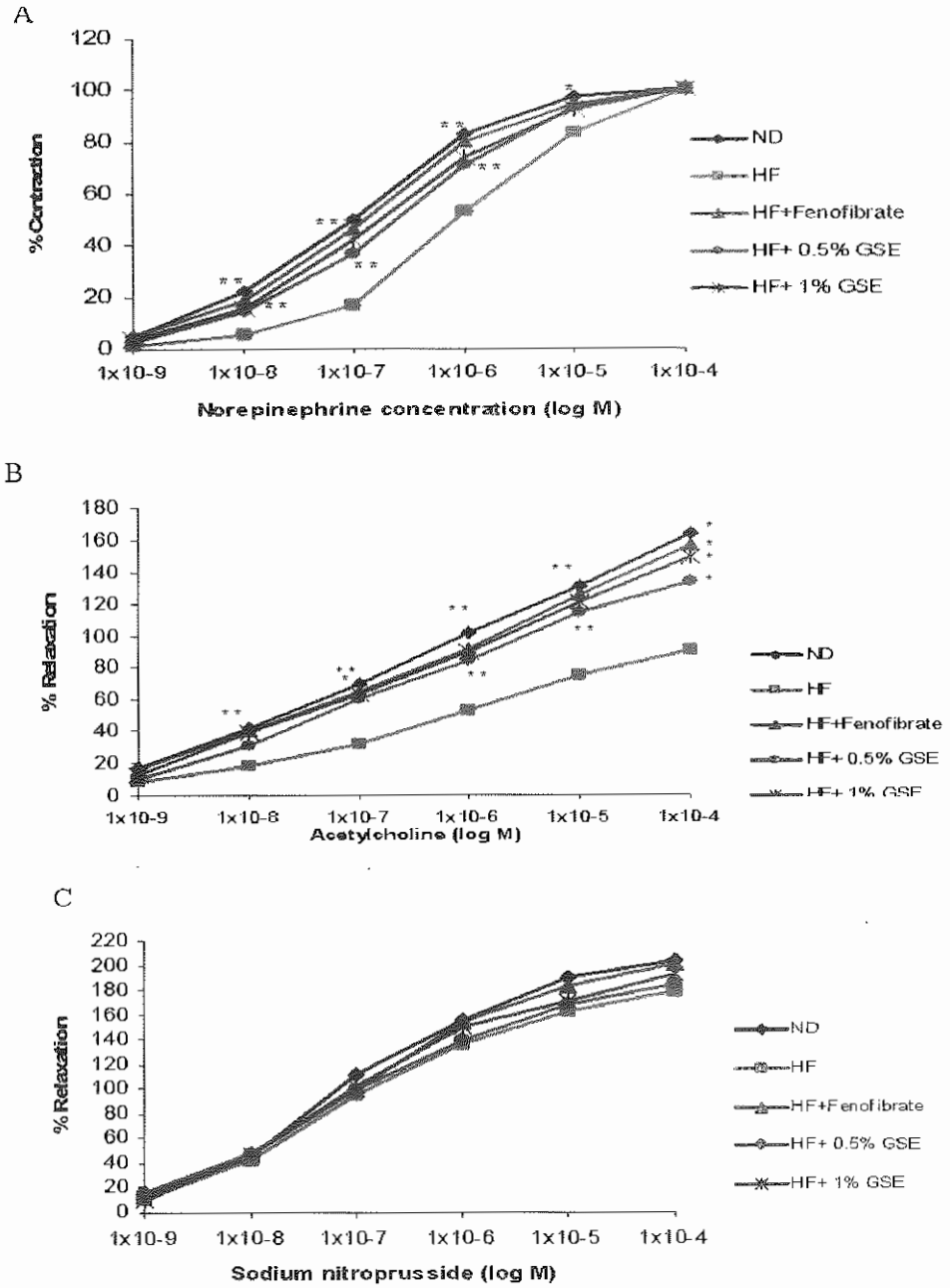

Figure 1. Contractile response to increasing doses of NE (A), endothelium-dependent relaxation in response to Ach (B) and endothelium-independent relaxation in response to SNP (C). All values are mean ± S.E.M. (n=8). * $P < 0.05$ compared to high fat diet group.

Discussion and Conclusion

The data presented in this study demonstrate that GSE has lipid lowering action and preserve normal function of vascular, in particular, endothelium-dependent vasodilation in hyperlipidemic rats induced by high fat diets. The development of risk factors induced by high fat diet is associated with endothelial dysfunction and a significant decrease in nitric oxide production in blood.

The results of this study indicate that dietary supplement of GSE may benefit patients with atherosclerosis by preserving endothelial functions through a mechanism related to its lipid lowering effect.

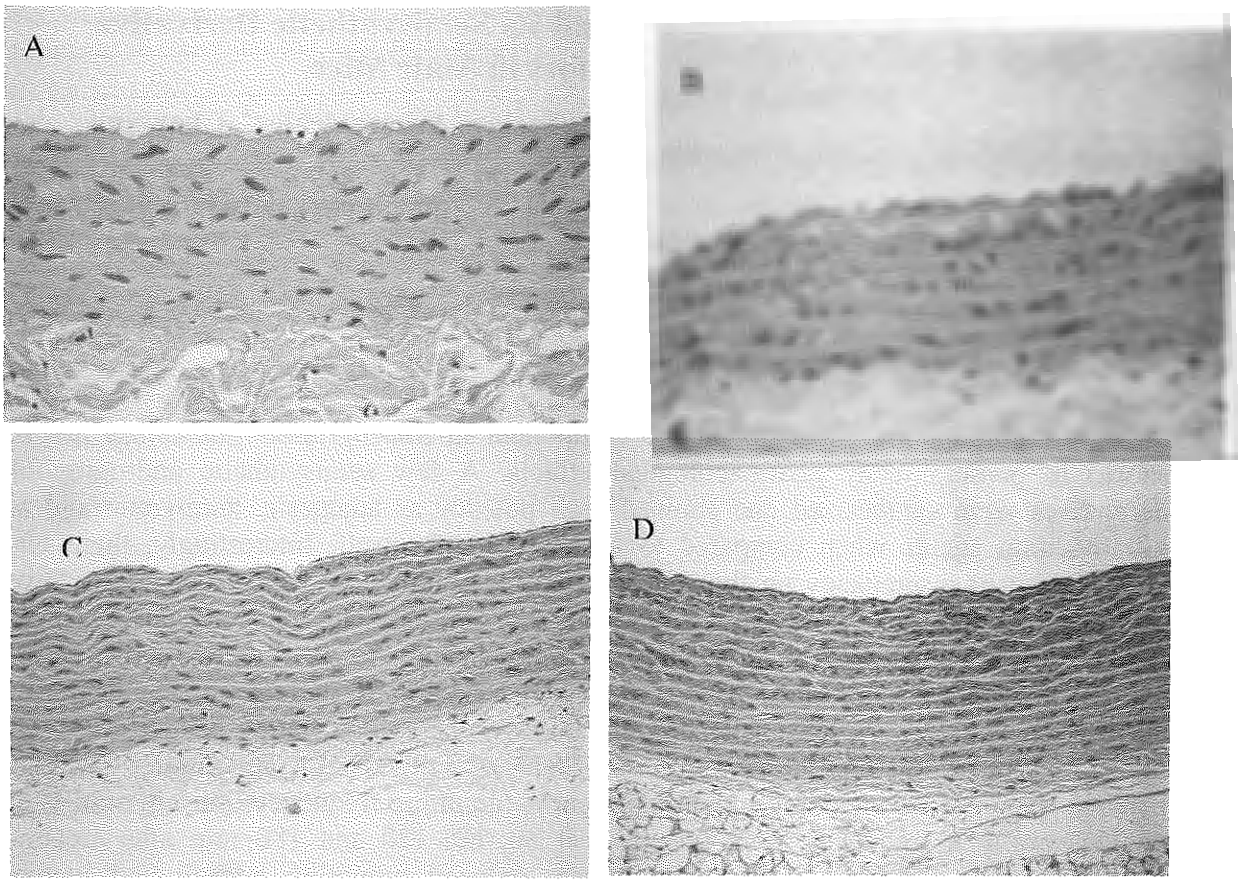

Figure 2. Representative aortic cross section from the five experimental groups showing H&E stained as following ND group (A), HF group (B), HF + fenofibrate group (C), HF + 1% GSE group (D) (x400).

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Original article **P08**

Effects of Andrographolide and Its Semisynthetic Derivative on Sexual Behaviors in Male Mice.

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Abstract

Andrographolide, a major active component in *Andrographis paniculata* has been shown to possess many pharmacological effects. A few semisynthetic andrographolide derivatives were synthesized and showed high potency as analgesic, antipyretic and anti-inflammation agents when compared to andrographolide itself. Interestingly, there was a large degree of discrepancy on the effect of andrographolide on sexual behaviors. Several studies, in animals or human, showed that *A. paniculata* may either have negative, no effect or positive effects. The aim of this study was to test for the effects of andrographolide (SS1) and its semisynthetic derivative (SS17) on sexual behaviors in male mice. The healthy and naïve female mice were artificially brought into estrous before mating behavior test. Male mice orally fed with either 50 mg/kg of SS1, SS17, or 5 mg/kg sildenafil citrate. Four sexual behavior parameters, including mounting latency, mounting frequency, intromission latency and intromission frequency were observed at 30, 60, 120 and 180 min after treatment. Treatment with sildenafil citrate significantly reduced mounting latency and increased mounting frequency from 30 to 180 min after treatment while reduced intromission latency at 120 min after treatment. SS1 significantly reduced mounting latency at 120 and 180 min and increased mounting frequency at 180 min after treatment. No effect on either mounting or intromission behaviors could be seen in SS17 treated mice. The results suggest that SS1 but not SS17 could probably enhance sexual behaviors in male mice.

Keywords: andrographolides, sexual behaviors, male mice.

Introduction

Andrographolide is a major component of *Andrographis paniculata*, which is one of the important herbal medicines, are widely used in China, India, Thailand and other Southeastern Asian countries. Andrographolide has been reported to have multiple pharmacological properties, such as analgesic, antipyretic, anti-inflammatory (1), and cardiovascular activities (2).

Several animal studies showed that *A. paniculata* may have contraceptive or anti-fertility effects following long-term treatment at high doses (20 mg/rat) (3). On the other hand, there was a large degree of discrepancy in the results, with some studies demonstrating no untoward effects even at the 1000 mg/kg dose (4). Moreover, in a phase I clinical study, no significant negative effect of *A. paniculata* (fixed combination "Kan Jang®") on male semen quality and fertility could be observed, and rather, the comment by the principally of volunteers was feeling of enhanced sexual potency during masturbation (5).

Recently, a few semisynthetic andrographolide derivatives were synthesized. A few of them showed high potency as analgesic, antipyretic and anti-inflammation agents when compared to andrographolide itself (1). Since, andrographolide had a few scientifically

studies for their effects on sexual functions, in this study andrographolide and its semisynthetic derivative, SS17, were tested for the effects on sexual behaviors in male mice.

Methods:

In these experiments, andrographolide (SS1) and its semisynthetic derivative (SS17) used in this study were received from Faculty of Pharmaceutical Sciences, Khon Kaen University. The healthy and naïve female mice used in the mating behavior test were artificially brought into estrous by subcutaneous injections of β-estradiol-3-benzoate in corn oil (10 μg/mouse) and progesterone in corn oil (500 μg/mouse) 48 hr and 4 hr, respectively, before experiment(6). SS1 and SS17 were suspended in corn oil, while sildenafil citrate was suspended in normal saline, all of them given orally to male mice at a dose of 50, 50 and 5 mg/kg, respectively. Sexual behavior test (7) in 10 min period was done at 30, 60, 120 and 180 min after treatment. The test was made by introducing estrous female mouse into the cage of treated male mouse during which the animal behaviors were recorded by video camera. Sexual behaviors were determined as: mounting latency (ML), time elapsed from introduction of male with female to the first mount; mounting frequency (MF), number of mounts; intromission latency (IL), time from the introduction of the female up to the first intromission by the male; and intromission frequency (IF), number of intromissions.

Results:

Effects on mounting latency: The effects of SS1, SS17 and sildenafil citrate on mounting latency at various times after treatment (Fig.1). Significant reduction in mounting latency, compared to control, could be observed in mouse treated with sildenafil citrate 5 mg/kg orally at 30, 120 and 180 min after treatment. Mice fed with 50 mg/kg SS1 showed a significant reduction in mounting latency, compared to control, at 120 and 180 min after treatment. Although there was a tendency of a reduction in mounting latency in mice received SS17, no significant effect could be seen.

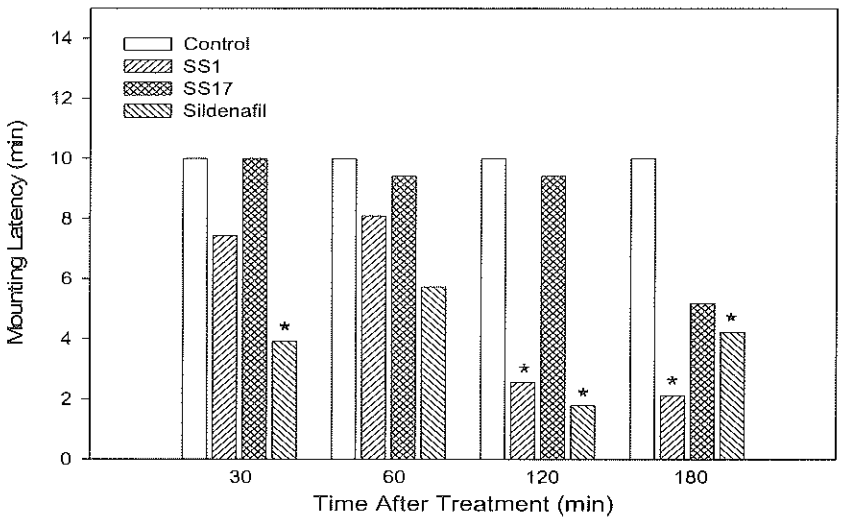

Figure 1. Effects of SS1, SS17 and sildenafil citrate on mounting latency in male mice.
* p value less than 0.05 when compared to the control at the same time point.

Effects on mounting frequency: The effects of SS1, SS17 and sildenafil citrate on mounting frequency at various times after treatment (Fig.2). Significant increase in mounting frequency, compared to control, could be observed in mouse treated with sildenafil citrate 5 mg/kg orally at 30, 120 and 180 min after treatment. Mice fed with 50 mg/kg SS1 showed a significant increase in mounting frequency, compared to control, at 180 min after treatment. No significant effect of SS17 on mounting frequency could be seen.

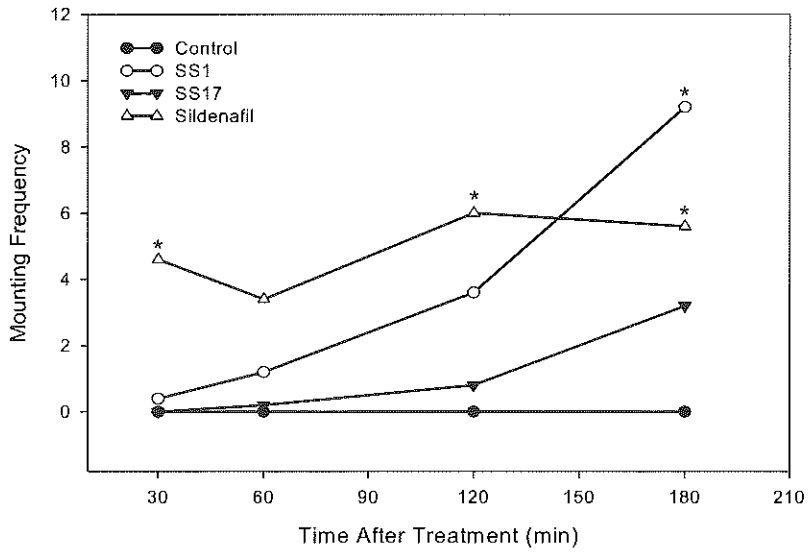

Figure 2. Effects of SS1, SS17 and sildenafil citrate on mounting frequency in male mice.
* p value less than 0.05 when compared to the control at the same time point.

Effects on intromission frequency: No effects of SS1, SS17 or sildenafil citrate on intromission latency could be observed (data not shown).

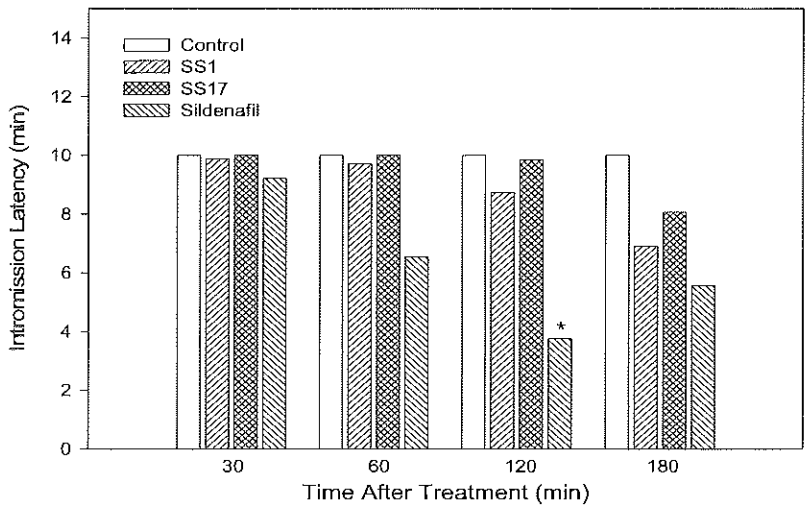

Figure 3. Effects of SS1, SS17 and sildenafil citrate on intromission latency in male mice.
* p value less than 0.05 when compared to the control at the same time point.

Effects on intromission latency: The effects of SS1, SS17 and sildenafil citrate on intromission latency at various times after treatment (Fig.3). Significant decrease in intromission latency, compared to control, could be observed in mouse treated with sildenafil citrate 5 mg/kg orally only at 120 min after treatment. Mice fed with 50 mg/kg of either SS1 or SS17 showed no significant effect on intromission latency.

Discussion:

From this study, it was shown that SS1 (andrographolide), an active substance from *Andrographis paniculata*, at a dose of 50 mg/kg orally in male mice, could probably have a positive effect on sexual behaviors as seen in decreasing mounting latency, increasing mounting frequency and decreasing intromission latency. No effect could be seen with SS17, a semisynthetic derivative of SS1, at a dose of 50 mg/kg orally in male mice. Interestingly, it was recently reported that SS17 was comparable and/or more potent than SS1 in analgesic, antipyretic and anti-inflammatory actions (1). It might be possible that the effect on sexual

behaviors of andrographolide is based on different mechanism from analgesic, antipyretic and anti-inflammatory actions, since many biological actions of andrographolide have been suggested (3). Further studies are needed to clarify the effects and the mechanism of actions.

Conclusion:

Andrographolide, but not a semisynthetic (SS17), seemed to possess the stimulatory effects on sexual behaviors in male mice. The effects were seen as the decrease in mounting and intromission latency and increase in mounting frequency.

Acknowledgements:

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Original article **P09**

Alteration of the Insulin Signaling Pathway by Grape Seed Extract in High Fructose-Fed Rats

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Abstracts

Grape seed extract (GSE) has been reported to have a broad spectrum of biological, pharmacological and therapeutic activities. The present study was to investigate the effect of GSE on plasma glucose, lipid profiles and insulin signaling pathway in high fructose-induced diabetic rats. The results showed that a high-fructose diet supplemented with GSE significantly decreased plasma glucose and triglyceride concentration ($p<0.05$). There were no significant differences in plasma cholesterol between the group supplemented with GSE and high fructose group. Moreover, GSE did not change the protein expression of insulin receptor whereas the group supplemented with 0.5%GSE slightly ameliorates the deficient expression of glucose transporter 4 (GLUT4) in high-fructose diet group. From this point of view, GSE is a feasible therapeutic strategy for prevention and treatment of patients with type 2 diabetes mellitus.

Keywords: Grape seed extract, Insulin signaling, High-fructose diet

Introduction

Type 2 diabetes is metabolic disorder found 90-95 % of all diagnostic diabetes patients, characterized by abnormal of carbohydrate and lipid metabolism.and associated with defection of insulin action on the peripheral target tissues. This defect causes high level of plasma glucose in circulation, whereas insulin level is normal or high (1).

Grape seeds, polyphenolic-rich sources (2), have continuously studied to their health beneficial properties. Grape seed extract (GSE) have been shown the broad spectrum of biological activities against free radicals scavengers, anticancer, anti-inflammatory and antibacterial activities (3), as well as being cardioprotective agent (4). The previous study was evaluated antihyperglycemic effect of GSE in diabetic rats (5), however, there is no any report demonstrated the effect of GSE on insulin signaling pathway in fructose-induced diabetic rats. Therefore, the study was to investigate the effect of GSE on plasma glucose, and lipid profiles in high fructose-induced diabetic rats. The study also determined the expression of proteins associated in insulin signaling pathway in diabetic rats.

Materials and methods

Animal models: Male Spraque Dawley rats (100-150g) were divided into five groups and received the experimental diet for 8 weeks. The first group received standard diet as the

control group. Group 2 received high-fructose (HF) diet (63% diet). Group 3, and 4 received high-fructose (HF) diet supplemented with 0.5% and 1 % (w/w) of GSE, respectively. The last group received high-fructose diet and orally received rosiglitazone (4 mg/kg) at the 5th week of the experimental period. The blood was collected to determine plasma glucose and lipid profiles at the end of experiment. The soleus muscles were removed and immediately freeze with liquid nitrogen and store at -80°C until homogenizing process.

Tissue homogenization: The soleus muscles were weighed and homogenized in 0.8 ml homogenizing buffer. The homogenate was then centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was used to determine protein concentration using Bradford assay and stored at -80°C until subjected to perform the western blot analysis.

Western blot analysis: 35 µg protein was used to run SDS-PAGE (10% separating gel) and then performed electrotransfer to PVDF membrane. The membrane was incubated with primary antibody against insulin receptor β or glucose transporter 4 (GLUT4) at 4°C overnight and then second antibody at room temperature for 1 h. After washing, blotted proteins were visualized using the enhanced chemiluminescence detection system (ECL).

Statistical analysis: Data are expressed as the mean ± SEM. Statistical differences among groups were determined using one-way analysis of variance (ANOVA). The LSD post-hoc comparisons were used to analyze the source of significant differences. A p-value< 0.05 was considered statistically significant.

Results

At the end of 8-week diet period, the high-fructose diet group significantly increased plasma glucose, cholesterol and triglyceride levels when compared to the control group.

The high-fructose diet group supplemented with 1 % GSE or rosiglitazone significantly decreased plasma glucose level when compared to high-fructose diet group ($P < 0.05$) (Table1).

Table 1. The effect of GSE on plasma glucose, lipid profiles in normal rat and high fructose-induced diabetic rats.

Parameters (mg/dl)	Control	HF	HF + 0.5%GSE	HF + 1%GSE	HF + Rosiglitazone
Plasma glucose	128.7 ± 14.2	165.4 ± 15.8[a]	139.4 ± 5.9	132.6 ± 11.4[b]	130.5 ± 7.1[b]
Total cholesterol	86.0 ± 2.5	99.2 ± 2.1[a]	99.4 ± 3.0[a]	98.6 ± 2.7[a]	91.0 ± 3.0[b]
HDL cholesterol	75.0 ± 3.3	81.9 ± 3.5	80.0 ± 3.2	80.0 ± 2.2	74.5 ± 3.2
Triglyceride	44.8 ± 3.7	76.7 ± 9.2[a]	59.2 ± 4.3[b]	57.2 ± 3.1[b]	46.8 ± 4.5[b]

Values are mean ± SEM (N=6), [a]p<0.05 compared to control group, [b]p<0.05 compared to high-fructose group.

There were no significant differences in plasma cholesterol and HDL between group treated GSE and rosiglitazone and high fructose diet group. Moreover, the group supplemented with 0.5% and 1%GSE significantly reduced plasma triglyceride when compared to high fructose diet group.

The expression levels of insulin receptor β (IRβ) and glucose transporter 4 (GLUT4) in rat muscles are shown in Figure 1 and quantification is summarized in Table 2. The protein expression of IRβ and GLUT4 in high-fructose diet group was significantly lower than normal diet group. The group supplemented with GSE did not alter the protein expression of IRβ (Figure 1A) whereas the expression level of GLUT4 in the group supplemented with 0.5%GSE slightly increased when compared to high-fructose diet group (Figure 2A).

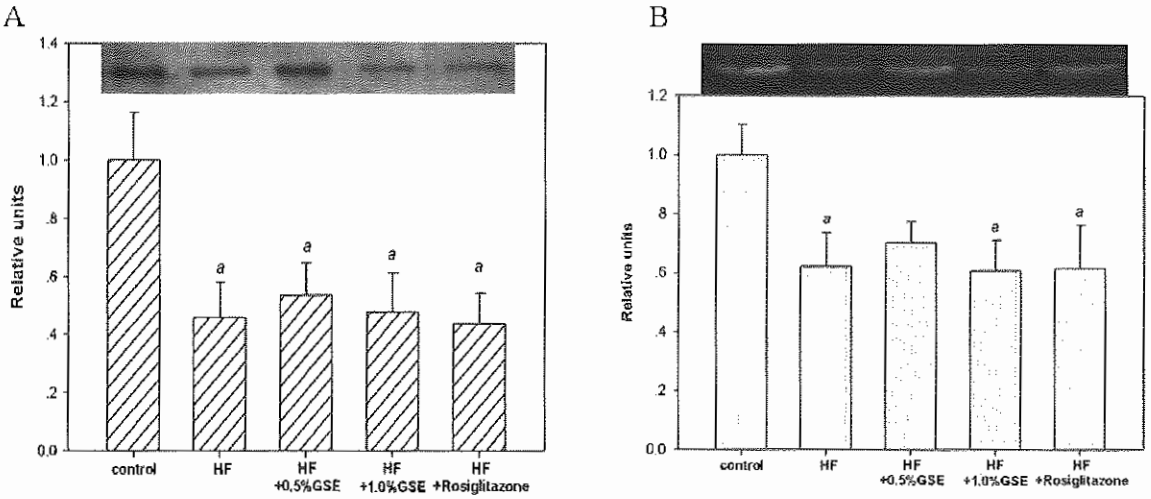

Figure 1. The effect of GSE on protein expression of insulin receptor β (A) and glucose transporter 4 (GLUT4)(B). [a]$p<0.05$ compared to control group; n= 3-4.

Table 2. Quantification of insulin receptor β and glucose transporter 4 (GLUT4) expression.

Relative units	Control	HF	HF+0.5%GSE	HF+1%GSE	HF+Rosiglitazone
IRβ	1.00±0.16	0.46±0.12 [a]	0.54±0.11 [a]	0.48±0.13 [a]	0.44±0.11 [a]
GLUT4	1.00±0.26	0.62±0.12 [a]	0.70±0.07	0.61±0.10 [a]	0.61±0.15 [a]

Values are mean ± SEM, [a]$p<0.05$ compared to control group.

Conclusion

Grape seed extract ameliorates hyperglycemia and hypertriglyceridemia in high-fructose-diet-fed rats and also slightly increases the expression of GLUT4 which plays important role in insulin-stimulated glucose uptake.

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Original article **P10**

Study on an Anti-nociceptive of the Ethanolic Extract from the Leaves of *Clausena harmandiana* Pierre in Mice

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Abstract

The ethanolic extract of *Clausena harmandiana* Pierre was tested for an anti-nociceptive effect in mice. Three models using hot-plate, tail-flick and writhing test were evaluated. The results showed significant anti-nociceptive activity when compared to the control ($p<0.05$) group.

Keywords: *Clausena harmandiana* Pierre, anti-nociceptive

Introduction

Clausena harmandiana Pierre is a member of the Rutaceae family, which is more commonly known in Thai as Song faa dong. It has been used in folk medicine for relief of aches and fever. The isolation of five coumarins: clausarin, dentatin, osthol, xanthoxyletin, nordentatin and one alkaloid, heptaphylline was reported in 1984 (1). Further investigations resulted in the isolation of two novel carbazol alkaloids, 2-hydroxy-3-formyl-7-methoxycarbazole and 7-methoxyheptaphylline (2). Three pure compounds extracted from this plant; heptaphylline, dentatin and clausarin exhibited antiplasmodial activity against *Plasmodium falciparum* (3). However reports of bioactivities related to Song faa dong remain un-clarified. The aim of this study was to investigate the anti-nociceptive activity of the ethanolic extract from leaves of *C. harmandiana* Pierre in animal models.

Materials and methods

1. Plant material: The plant was collected from Khon Kaen province which is located in the Northeast of Thailand. A voucher specimen of *C. harmandiana* was identified by Mr. Supachai Tiyavorranun and was compared with the herbarium sample kept at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

The dried leaves of *C. harmandiana* was ground, macerated with ethanol and occasionally stirred at room temperature for 3 days. The solvent was then evaporated under reduced pressure, and the extract was concentrated and stored in a refrigerator until use.

2. Chemicals: The following substances were used: morphine hydrochloride, diclofenac, acetic acid, propylene glycol (PG).

3. Animals: All animals used in study were male ICR mice (30±5 g), obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhonpathom, Thailand. The animals were housed for at least one week in the animal room. They had free access to food and clean water. Animals were fasted for 6-8 h before the experiment but were allowed free access to water, and were allowed to acclimatise in the laboratory for at least 1 h before testing.

4. Assessment of anti-nociceptive effect

Hot plate test: Mice were placed on a hot plate maintained at 55±1°C, latency of nociceptive response such as licking of the hind limb or jumping was measured. Each mouse was tested to measure a baseline response prior to administration of the drug. The mice were divided into 3 groups; control (PG, given by oral route), standard drug (morphine, 10 mg/kg, subcutaneously) and the test compound (extract of *C. harmandiana,* 500 mg/kg, orally). The tests were performed to obtain the reaction times at 15, 30, 60 and 90 min after administration. Six mice were used for each group in this test.

Tail flick test: A radiant heat was used to measure response latency. Mice respond to heat stimulation by flicking or removing tail. Each mouse was tested to obtain a baseline response prior to administration of the drug. The mice were divided to 3 groups as previously described; control, standard drug (morphine 10 mg/kg) and tested compound (extract of *C. harmandiana* 500 mg/kg). Test reaction times were taken at 15, 30, 60 and 90 min after each administration. Six mice were used for each group in this test.

Writhing test: Solution of 1% acetic acid was injected intraperitoneally into mice (100 mg/kg). Writhing behavior such as the numbers of abdominal constriction and stretching were counted. The extract of *C. harmandiana* Pierre at doses of 250, 500 and 750 mg/kg were given orally to the test group. The control group was PG, and the standard drug group was diclofenac given subcutaneously at a dose of 20 mg/kg. Drugs were administered 20 minutes before acetic acid injection.

5. Statistical analysis: The statistical analyses was performed by one-way ANOVA (SPSS version 11.5)

Results

Hot plate test

Morphine at dose 10 mg/kg s.c. and the ethanolic extract of *C. harmandiana* at the doses of 500 mg/kg po. showed an increase in the time of reaction to the thermal stimulus compared to control, with respective pain latency of 198.4 and 137.5% compared to control (Fig. 1) (% increase is calculated by using area under the curve: AUC, the area under the plot of the latency time and time after administration).

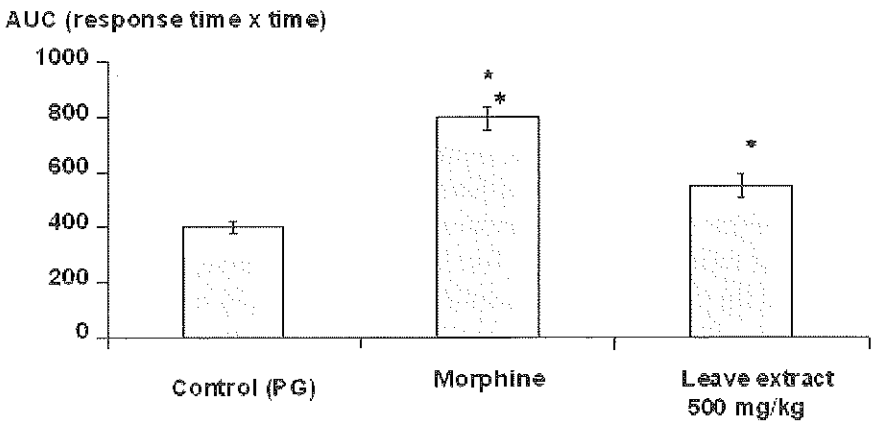

Figure 1: The effect on nociceptive response induced by hot plate in mice. Each value represents the mean ± SD (n=6); $^{*}p < 0.05$.

Tail flick test

Morphine at a concentration of 10 mg/kg (s.c) significantly increased the pain latency (123.0%), whereas the ethanolic extract of *C. harmandiana* (500 mg/kg) did not show significant difference when compared to the control group (Fig.2).

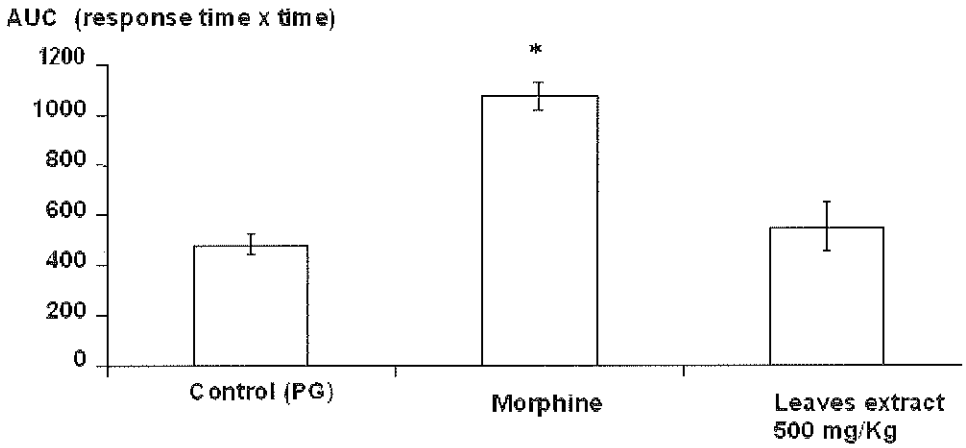

Fig 2: The effect on nociceptive response induced by heat (water bath) in mice of tail-flick test. Each value represents the mean ± SD (n=6); $^{*}p < 0.05$.

Writhing test

In the acetic-induced writhing response test, diclofenac at dose 20 mg/kg s.c. and the ethanolic extract of *C. harmandiana* Pierre at all doses tested (250-750 mg/kg) significantly inhibited writhing compared to the control ($p < 0.05$) (Fig. 3). The percentage of pain inhibitory effect in mice that were treated with diclofenac (20 mg/kg, s.c) was 49.0%, whereas the treated orally with ethanolic extract at concentrations of 250, 500 and 750 mg/kg were 33.0, 49.6 and 67.0 % respectively (% inhibition is calculated by using area under the curve. Different dose effects in the acetic-induced writhing response test showed a dose-dependent manner.

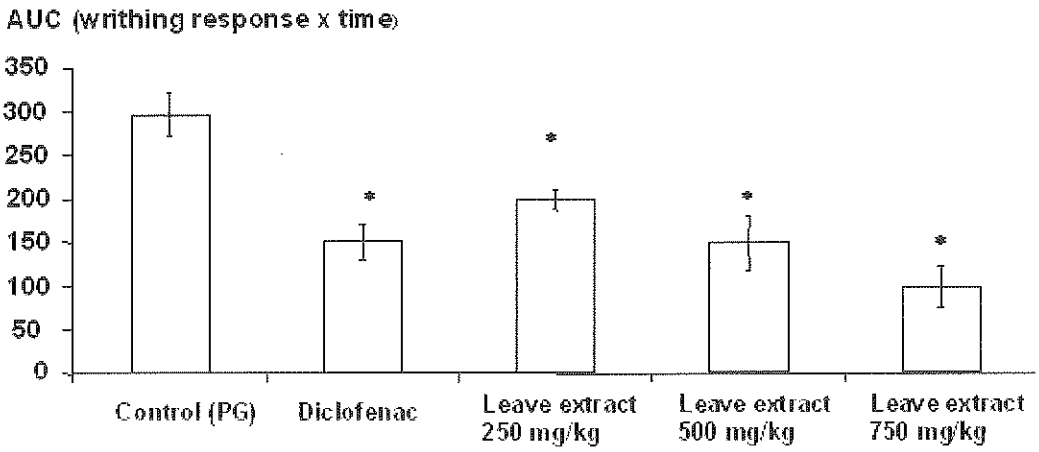

Figure 3: The anti-nociceptive activity of *C. harmandiana* on acetic acid-induced writhing response in mice. Each value presents mean ± SD (n=6); $^{*}p<0.05$.

Discussion

The hot plate and tail flick tests have been used for evaluation of centrally acting analgesic effect. The writhing test by acetic acid-induced abdominal writhing is the visceral pain model, whereas the process of release of arachidonic acid via cyclo-oxygenase and prostaglandin biosynthesis play an important role in the nociceptive mechanism (4,5). The extraction doses tested were shown to possess anti-nociceptive activity. The ethanolic extract of *C. harmandiana* at dose of 500 mg/kg significantly increase the latency time to the thermal stimulate in hot plate test but not in tail flick test. For the writhing test, the extract significantly inhibited writhing in dose dependent manner.

Conclusion

This study has shown that the ethanolic extract from leaves of *C. harmandiana* possess significant anti-nociceptive as shown by the effect on laboratory mice at the doses investigated. This result also supports the traditional use of this plant in that the ethanolic extract of *C. harmandiana* Pierre possesses anti-nociceptive activity via pheripheral and central pathway. In the future it may be worthwhile to investigate the pure compounds isolated from this plant.

Acknowledgement

The authors would like to thank to the Faculty of Pharmaceutical Sciences and the Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand, for the use of facilities and equipments throughout this project.

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Original article **P11**

Preliminary Study on Effects of a Standardized Extract of *Centella asiatica*, ECa 233, on Deficit of Learning and Memory induced by an Intracerebroventricular Injection of β-Amyloid Peptide in Mice

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Abstract

The purpose of this study was to primarily investigate the effects of the standardized extract of *Centella asiatica* (ECa 233) on cognitive deficits induced by an intracerebroventricular (i.c.v.) injection of β-amyloid peptide (25-35) into male ICR mice weighing 25-35 g. Seven days after β-amyloid peptide (25-35) injection, we examined their behavioral changes by Morris water maze test. In addition, locomotor activity tests was also performed. In comparison to sham mice, impairment of learning and memory was significantly observed in mice treated with β-amyloid peptide (25-35). Pretreatment by ECa 233 (10 mg/kg B.W., p. o., twice daily) for 7 days before the injection of β-amyloid and throughout experiment could significantly improve deficit in learning and memory, on spatial memory in Morris water maze test, however, ECa 233 did not alter locomotor activity. These results suggested that pretreatment of the standardized extract of *Centella asiatica* (ECa 233) is effective in ameliorating cognitive deficits induced by intracerebroventricular injection of β-amyloid peptide (25-35) in mice. Further investigation should be conducted to confirm the results observed as well as to shed light on its mechanism.

Keywords: Alzheimer's disease, Morris water maze

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in the elderly. The pathological hallmarks of AD are numerous including memory loss and decline in cognitive function, high level of brain oxidative stress as well as a deposit of senile plaques containing amyloid β peptide (Aβ) and neurofibrillary tangle in brain region (1). Aβ, a 39-43 amino acid hydrophobic peptide cleaved from amyloid precursor protein (APP), has been suggested to underlie oxidative stress and subsequently neuropathology observed in AD (2). Similarly, an intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ can induce oxidative stress as well as neurotoxicity resemble those found in AD in animal models (3). Thus, an intracerebroventricular (i.c.v) injections of $A\beta_{25-35}$ are useful models for understanding the pathogenesis of AD and its treatment. ECa 233 is a white to off-white standardized extract of *C. asiatica* containing triterpenoids not less than 80% and the ratio of madecassocide and asiaticoside would be kept at 1.5±0.5 (4). Previously, we have demonstrated ameliorating effects of orally given ECa 233 on learning and memory deficit in a transient bilateral common carotid artery occlusion (2VO) model in mice (4). Furthermore, in terms of acute toxicity and sub-chronic toxicity, ECa 233 was found to exhibit very favorable toxicological profiles. No death was observed in mice acutely treated with ECa 233 in the dose up to 10 g/kg and oral administration of ECa 233 in the doses of 10-1,000 mg/kg for 90 days did not cause any significant changes in all parameters observed. Therefore, it is of our interest to investigate further the effects of ECa 233 on cognitive deficits induced by the i.c.v. injection of $A\beta_{25-35}$ peptide in mice.

Materials and Methods

1. Preparation of the test compound

ECa 233 was prepared by Dr. Chamnan Patarapanich and co-workers, Faculty of Pharmaceutical Sciences, Chulalongkorn University. It was suspended in 0.5% carboxymethylcellulose (0.5% CMC) solution. The test substance, ECa 233 or 0.5% CMC, was orally administered by gavage tube twice daily. $A\beta_{25-35}$ (Sigma) was dissolved in sterile double distilled water at a concentration of 1 mg/ml. The $A\beta_{25-35}$ (1 mg/ml) was aggregated, or 'aged' by incubation in sterile distilled water at 37°C for 4 days. Light microscopy was used to assess the formations of birefringent fibril-like structures and globular aggregates of aged $A\beta_{25-35}$ (5).

2. Animals

Male ICR weighing 25-35 g. obtained from National Laboratory Animal Centre at Salaya campus, Mahidol University, Nakornpathom, Thailand were housed in groups for four to five mice under controlled environmental conditions of a 12- h light/ dark cycle at 25±2°C. After acclimatization, the mice were randomly divided in to three groups as followed; one sham-operated group (receiving 0.5% CMC) and two $A\beta_{25-35}$ treated groups (one received 0.5% CMC and the other received ECa233,10 mg/kg B.W., p. o., in 0.5% CMC)

All experiments reported herein were conducted with an approval of Ethical committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3. Experimental protocol

Animals were divided randomly into three groups of 7 animals each. $A\beta_{25-35}$ (9 nmol/ mouse) dissolved in sterile double distilled water was prepared and being injected intracerebroventricularly into mice at day 0. ECa 233 was administered orally (10 mg/kg, B.W., p. o., twice daily) for 7 days before an injection of $A\beta_{25-35}$ and continued throughout the experiment. Morris water maze (MWM) test in which the escape latency (time lag for the animal to find the hidden platform) was conducted for 5 days successively and the probe trial was conducted on day 6. At the end of experiment locomotor activity was measured using activity cage (UGO Basile, Comerico, Italy).

4. Statistical analysis

Results are expressed as mean ±S.E.M. Statistical analysis was carried out by one-way analysis of variance. Tukey's HSD test were used for post hoc comparison with $p<0.05$ was considered to indicate statistical significance.

Results

Effects of ECa 233 on learning and memory deficit induced by $A\beta_{25-35}$ in Morris water maze test

As shown in Fig.1, $A\beta_{25-35}$ treated mice significantly demonstrated impairment in learning and memory in MWM task. Accordingly in probe trial on day 6, $A\beta_{25-35}$ treated mice were comparatively found to spend lesser time in the quadrant where the platform was removed than did the sham-operated group. ECa 233 (10 mg/kg) given 7 days before and after the administration of $A\beta_{25-35}$ significantly improved the learning and memory deficit induced by $A\beta_{25-35}$. Mice receiving ECa 233 (10 mg/kg, B.W., p. o., twice daily) demonstrated the escape latency on day 3 at 20.99 ± 6.36 sec. (Fig. 1A) and spend time in the quadrant longer than those exhibited by $A\beta_{25-35}$ treated mice receiving 0.5% CMC. In probe trial, the percentage of time spent in the quadrant were 35.57±1.77% for sham-operated group, 20.49±2.25% for $A\beta_{25-35}$ treated group and 30.78±2.26% for $A\beta_{25-35}$ treated group receiving ECa233 at 10 mg/kg, twice a day (Fig 1B).

Effects of ECa 233 on locomotor activity

By using an activity cage, locomotor activity of each group of the animals was measured. Neither the i.c.v. administration of $A\beta_{25-35}$ nor the oral administration of ECa 233 exerted any significant effects on locomotor activity of the mice (Fig.2)

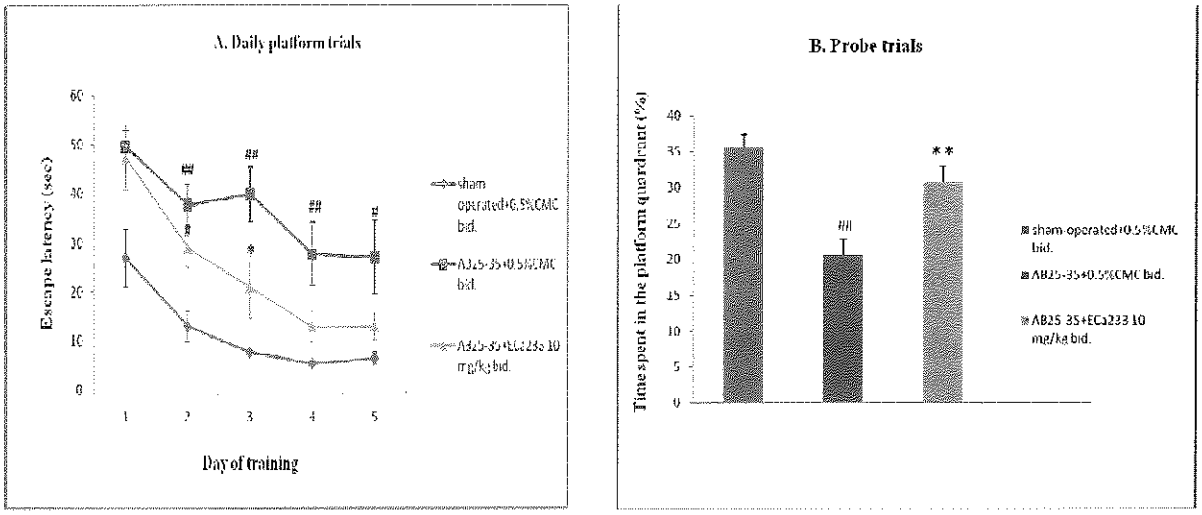

Figure 1 Effects of ECa 233 on learning and memory deficit induced by $A\beta_{25-35}$ in Morris water maze test. The hidden platform trials (A) were carried out on day 7-12 after the start β - amyloid peptide (25-35) injection. The probe trials (B) was carried out on day 12 after the β - amyloid peptide (25-35) injection. Each data point represents the mean ± S.E.M. each from 7 mice. $^{\#\#}$ P<0.01 and $^{\#}$P<0.05 denotes significantly different from sham-operated group. ** P<0.01 and * P<0.05 denotes significantly different from β - amyloid peptide (25-35) injected group.

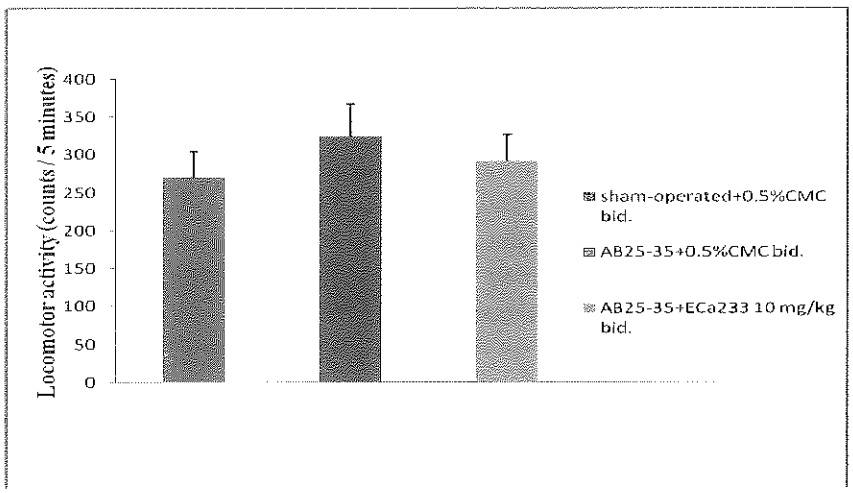

Figure 2. Effects of ECa 233 on locomotor activity. Bars represent mean ± S.E.M., each from 7 mice (n=7).

Discussion and conclusion

Intracerebroventricular infusion of $A\beta_{25-35}$, $A\beta_{1-40}$ and $A\beta_{1-42}$ have been previously reported to induce learning and memory impairment in animals (6,7,8). In line with them the present study demonstrated that intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ induced learning and memory deficits in Morris water maze task. On day 3, mice treated with $A\beta_{25-35}$ spent much longer time to find the platform than did the sham-operated group. Oral administration of ECa 233 10 mg/kg for 7 days prior to the injection of $A\beta_{25-35}$ and being continued throughout the experiment was found to significantly shorten the escape latency of $A\beta_{25-35}$ -treated mice. The ameliorating effect of ECa 233 was also further confirmed in the probe trial. Participation of stimulation or inhibition of motor activity that might affect the results observed in MWM test was excluded by the finding that ECa 233 showed no effect on locomotor activity (Fig.2). Our findings hereby clearly support the positive effect of ECa 233 on impairment of learning and memory in 2VO model previously reported (4). Though it is highly speculated that anti-oxidation property of ECa 233 seen as a reduction of the brain level of malondialdehyde in 2VO model may underlie the effects observed in the present study, it is challenging to get the proof. Further investigation should be made to strengthen the results observed and in addition, to conclude the mechanisms involved. In consideration to the unmet need for medication to alleviate dementia in the elderly which is the fast growing population in our country, extensive studies on ECa 233 which is a standardized extract of *C. asiatica* with well defined characteristics and very favorable safety profiles are obviously needed.

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Original article **P12**

Vascular Endothelial Dysfunction and Arterial stiffness in HbE- β-thalassemia/ patients

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Abstract

Thalassemias are anemias of variable severity, resulting from mutations of the genes encoding the synthesis of α and β globin chains of hemoglobin. Serious complications from thalassemia are associated with iron overload with consequences of oxidative organ damages including cardiovascular and endocrine dysfunctions. We investigated the relationship of oxidant status and cardiovascular function in pediatric patients with thalassemia. Fifteen blood transfusion-dependent β-thalassemia and hemoglobin E (HbE-β-thal) patients and 15 aged matched healthy controls were recruited in the study. Oxidant status was determined, peripheral arterial elasticity was analyzed by measurement of brachial-radial pulse wave velocity (brPWV) and vascular endothelial function was assessed by a forearm blood flow technique. Patients showed a sign of iron overload, where non-transferrin bound iron (NTBI) was significantly increased in patients compared with control subjects. Oxidative stress was increased in the patients, as plasma lipid peroxidation and protein carbonyl were increased and blood glutathione (GSH) levels and ratio of GSH to GSH disulfide were reduced. Forearm vasodilatory response to reactive hyperemia, a measurement of vascular endothelial function, was significantly decreased in patients compared with healthy controls. However, brPWV was not different between the two groups. Serum NTBI was correlated with oxidant parameters and forearm blood flow. It was concluded that thalassemia pediatric patients showed iron overload which was associated with oxidative stress and vascular endothelial dysfunction.

Keywords: Thalassemia, Non-transferrin bound iron, Oxidative stress, Forearm blood flow, Pulse wave velocity

Introduction

Thalassemias are common monogenic disorders of hemoglobin synthesis. In β-thalassemia, there is partial or complete lack of β-chain synthesis. The remaining excess of unpaired α-hemoglobin chains undergoes denaturation and auto-oxidation, contributing to increased oxidants, ineffective erythropoiesis, hemolysis and shortened erythrocyte survival (1). Hemoglobin E -β-thalassemia (HbE-β-thal) is the most common hemoglobin variant in Southeast Asian populations and variably characterized from a mild to severe transfusion dependent thalassemia major. Regular blood transfusions in thalassemia can result in excessive iron overload and iron-induced oxidative stress, which leads to increase organ damages, particularly cardiovascular and endocrine systems (2). It has been reported that enhancement of iron overload may cause left ventricular systole and diastole dysfunctions, decrease arterial elasticity and impair vascular endothelial function in adult thalassemia

patients (3). These cardiovascular complications are the leading cause of mortality in thalassemia. It is interesting to determine if thalassemia presents any signs of changes in vascular function in young patients who have been exposured to iron overload for a relatively short period of time, as endothelial dysfunction and arterial stiffness are early manifestations of most cardiovascular complications. In this study, we evaluate oxidant status, vascular endothelial function and arterial elasticity in pediatric patients with HbE-β-thal.

Material and Methods

1. Subjects Fifteen pediatric patients with transfusional dependent HbE-β-thal and 15 healthy age-matched subjects were recruited in the present study. Subjects were given an explanation regarding the study and written consents were obtained from all parents. The study protocol has been approved by the Khon Kaen University Ethics Committee for Human research.

2. Assay of oxidant parameters Plasma malondialdehyde (MDA) was assayed as thiobarbituric acid reactive product (TBAR) according to an established method. Glutathione (GSH) and glutathione disulfide (GSSG) in the blood were determined by a previously described method using 1-methyl-2 vinyl-pyridinum trifate (M2VP) as a glutathione scavenger (4).

3. Assay of non - transferrin bound iron Non- transferrin bound iron assay was modified from a previous described method (5). An aliquot of 200 µl of serum ultrafiltrate was diluted 1:2 (v/v) with 0.50 M HEPES buffer (pH 7.0). Fifty µl of 0.15 M thioglycolate, and 50 µl of 0.05 M bathophenanthrolinedisulfonic sodium (BPT), a chromogen for iron (II), were added to the solution for colorimetric measurement of the Fe (II)-BPT complex at absorbance of 537 nm.

4. Assay of Forearm Blood Flow Forearm blood flow (FBF) was measured by venous occlusion plethysmography, following subjects rested in a supine position for 15 min. A mercury-filled silastic tube was used to assess the forearm blood flow following our recent described report (4). The FBF after a brief ischemia of the forearm represented forearm vasodilatory response was expressed as the percentage change of FBF from baseline to the maximum flow during reactive hyperemia.

5. Assay of arterial pulse wave velocity The brachial-radial pulse wave velocity (brPWV), an indicator for peripheral arterial stiffness, was measured by using the arterial compliance monitor equipment (Model GDVCM/2A, Barts and the London, UK), previously validated (6)). After resting in lying supine, arterial PWV was measured by placing a photo-plethysmographic pulse sensing probe over the brachial artery and a pulse oximetry probe over the middle finger. The pulse waveforms obtained from both probes were analyzed off-line by custom-written software which measures the time delay between the feet of the proximal and distal pluse waves. The PWV was calculated from distance and time delay as meter/s. The brPWV was also determined during a reactive hyperemia following a brief occlusion of the upper arm.

6. Statistical Analysis Data are present as mean ± SD. Comparison of measured variables between patients and controls were made by Student's t test. The relationship of 2 variables was analyzed by Pearson's correlation.

Results

The characteristics of the subjects are showed in Table 1. Hemoglobin levels of the patients in pre- transfusion period were lower than healthy controls. Patients had significantly increased heart rate, but normal blood pressure when compared with controls (Table 1).

Oxidant status in the blood of patient group was increased, as lipid peroxidation and protein carbonyl were significantly increased (Table 2). Antioxidant capacity, measured as total GSH level and ratio of reduced GSH to GSSG, was lower in thalassemia patients. The labile serum iron, as measured as non-transferrin bound iron, was significantly increased in thalassemia patients and it was correlated with GSH, MDA and protein carbonyl, r= -0.45, 0.70 and 0.62, with $p<0.01$, respectively.

Table 1 Characteristics and Hemoglobin data of healthy controls and Thalassemia subjects.

	Controls (n=15)	Thalassemia subject (n=15)
Gender, male/female	5/10	8/7
Age (yr)	15.2 ± 0.8	13.6 ± 1.7*
BMI	22.2 ± 3.6	16.7 ± 2.0*
Heart rate (bpm)	71.4 ± 9.7	88.9 ± 9.2*
Systolic pressure (mmHg)	104.5 ± 10.2	102.9 ± 12.6
Diastolic pressure (mmHg)	62.2 ± 7.5	59.3 ± 8.0
Hemoglobin (g/dl)	13.3 ± 1.9	7.8 ± 0.7*

Data are mean ± SD. * $p<0.05$ vs healthy controls

Table 2 Parameters of antioxidant, oxidant status, NTBI and pulse wave velocity in controls and HbE-β-Thal subjects.

	Controls (n=15)	Thalassemia subject (n=15)
Total glutathione (μM)	599.8 ± 151.1	412.0 ± 120.5*
Redox ratio of GSH/GSSG	63.7 ± 39.2	37.4 ± 15.4*
Lipid peroxidation (μM)	2.8 ± 0.68	4.3 ± 1.5*
Protein carbonyl (nmol/mg protein)	6.3 ± 2.4	10.0 ± 2.5*
Non-transferrin bound iron (μM)	0.51 ± 0.43	3.2 ± 1.1*
Basal brPWV (m/s)	9.53 ± 2.1	8.7 ± 2.6
brPWV during hyperemia (m/s)	7.0 ± 2.3	6.4 ± 1.7

Data are mean ± SD. * $p<0.05$ vs healthy controls

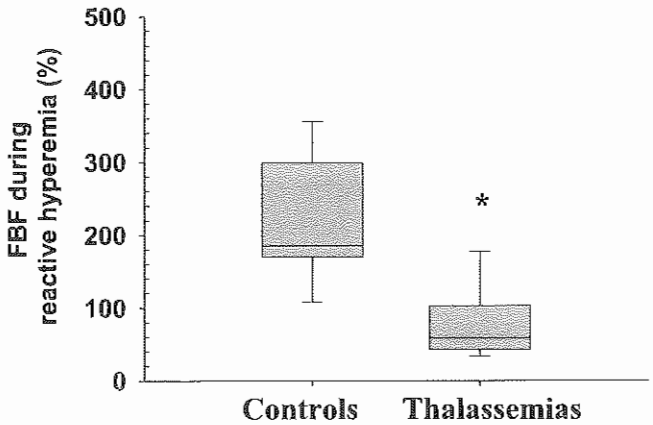

Figure 1 Forearm blood flow response to reactive hyperemia, shown as % increase from basal FBF. Line in the boxes represent median and 95% confident intervals, * Significant difference form control group, $p< 0.05$.

Forearm blood flow (FBF) was determined for assessment of vascular endothelial function. Forearm vasodilatory response to reactive hyperemia was markably decreased in patients compared with controls (Figure 1) and that was well correlated with NTBI (r= -0.64,

$p<0.001$). However, the basal brPWV and brPWV during reactive hyperemia in the young patients were not different from healthy controls (Table 2).

Discussion

Our young thalassemia patients presented with increased oxidative status and increased labile serum iron, despite of regular iron chelation therapy with desferioxamine. These changes were associated with vascular endothelial dysfunction as assessed by the FBF.

The non-transferrin bound iron is redox active and has potential to cause oxidative stress and oxidative damage (7). Increased oxidative status with depletion of antioxidant have been reported (4, 7). The present study also shows a relationship of the levels of labile iron with oxidative status. This change in body oxidants, in particularly NTBI, may be causally related to vascular endothelial dysfunction. The endothelial dysfunction is presented as an early biomarker and also indicator for vascular functional changes, which may lead to a number of cardiovascular complications. Arterial stiffness is apparent following alterations of arterial structures with increased intima-media thickness (8). The brPWV did not change in current study. This may imply that there might be no obvious structural changes in the patients, as the alterations may be apparent after chronic exposure to iron overload. Moreover, the brPWV measurement may be not sufficiently sensitive to detect a subtle change in these young patients. In conclusion, our findings suggest that measurements of early cardiovascular markers may be useful as a therapeutic target for prevention of cardiovascular complications in young thalassemia patients.

Acknowledgments

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Original article **P13**

Effect of Grape Seed Extract on Lipid Peroxidation and Brain Damage Induced by High-Fat Diet in Rats.

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Abstract

Lipid peroxidation by reactive oxygen species (ROS) is known to be involved in the damaging process of several neurodegenerative diseases. The present study was designed to determined the neuroprotective effect of grape seed extract (GSE) on oxidative stress and brain damage induced by high-fat diet in rats. Comparing to the untreated high-fat diet rats, feeding with high-fat diet containing GSE 0.5 and 1% to healthy male Wistar albino rats for 8 weeks resulted in a significant reduction in lipid peroxidation marker, malondialdehyde and nitric oxide in brain. Pathological investigation demonstrated also the decreasing of dark neurons occurred in cerebral cortex and hippocampus regions of the GSE treated rats comparing to the high-fat groups. Results of the present study suggest that GSE showed its efficacy for brain protection from lipid peroxidation induced by high-fat diets

Keywords: Lipid peroxidation, Grape seed extract, High-fat diet, Brain

Introduction

Oxygen and nitrogen free radicals play a crucial role in the pathophysiology of several brain disorder. Increasing of radicals production has been detected in animal brains during cerebral ischemia. GSE could reduce this increase by suppression of lipid peroxidation and reduced brain injury in neonatal rats (1). GSE contain a number of polyphenols including proanthocyanidins and procyanidins and are powerful antioxidant. It was recently reported that GSE could reduce neuronal damage in the adult gerbil after 5 min of forebrain ischemia, suppressed lipid peroxidation and reduced hypoxic ischemic brain injury (2).

Brain tissue is very sensitive to action of free radicals because there are high concentrations of polyunsaturated fatty acids and iron, both of which actively generate free radicals. Furthermore, hypercholesterolemia has been reported to increase superoxide anion production in endothelial cells and induced hypoxic ischemic brain injury(4). Therefore, the objectives of this study were to determine the neuroprotective effect of GSE on oxidative stress and brain damage induced by high-fat diet in rats.

Materials and Methods

GSE was obtained from waste products of the winery industry and prepared following a procedure previously described. Dried grape seed was powderized and extracted by water. Forty male Wistar albino rats, were randomly divided into 5 groups. Group1, normal diet (ND); group2, high-fat diet (HF); group3, high-fat diet and oral

administered with fenofribrate 100 mg/kg/day; groups 4 and 5, rats were fed high-fat diet containing GSE concentration 0.5% and 1.0%, respectively. After 8 weeks, all rats were sacrified, blood was collected by cardiac puncture and the brain was immediately removed from skull. Brains were sectioned sagitally as right and left hemispheres. Right hemisphere was removed and fixed with a buffered 10% formaline solution for pathological examination. Left hemisphere were wash and homogenized in iced-cold PBS buffer, centrifuge and clear supernatant was taken and used for assay of MDA, NO production and protein.

Statistical analysis

Datas were expressed as mean± SEM . Statistical analysis were carried out by analysis of variance followed by appropriate post-hoc tests including multiple comparision tests (LSD). A p-value less than 0.05 was considered statistically significant.

Results

As shown in Figure 1, GSE and fenofibrate treatment significantly reduced NO production and MDA level in brain tissue in which were markedly increased in high fat diet control rats. Comparing with the HF group, NO production in blood of GSE and fenofibrate treated rats were significantly increased but there were no statistically difference of blood MDA levels of all groups (Figure 2). The pathological investigation revealed scattered and grouped dark neurons in the cerebral cortex and hippocampus (Figure 3). The occurrence of dark neurons belong to each groups is shown in Table 1 which shows a lower degree of cell death in GSE treated rats comparing with the HF diet. There is no statistically significant difference between the normal diet and HF diet + GSE (0.5% and 1%) and fenofibrate-administered groups.

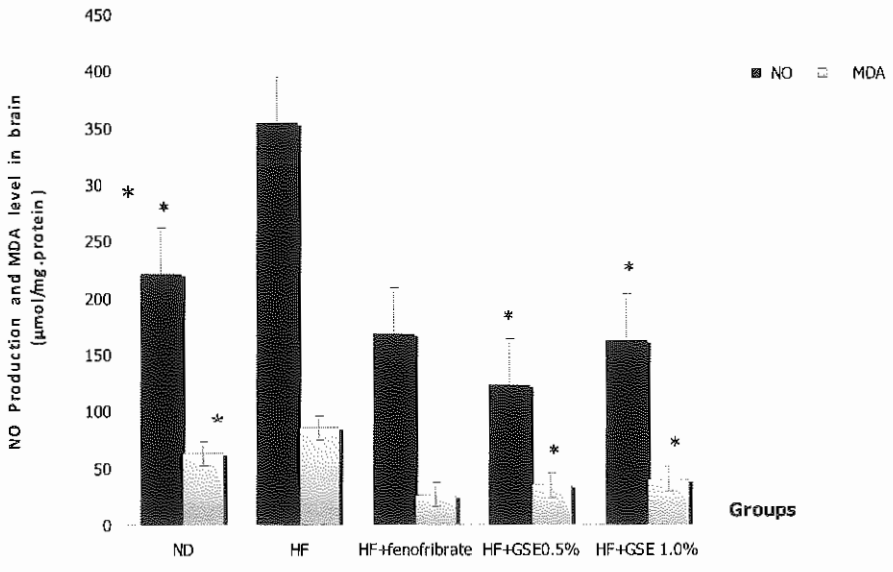

Figure 1 Effect of GSE (0.5% and 1%) on NO production and MDA levels in brain tissues of rats for each group. Each value represents the mean ± SEM of 8 rats.
* $p<0.05$ vs. HF-diet group.

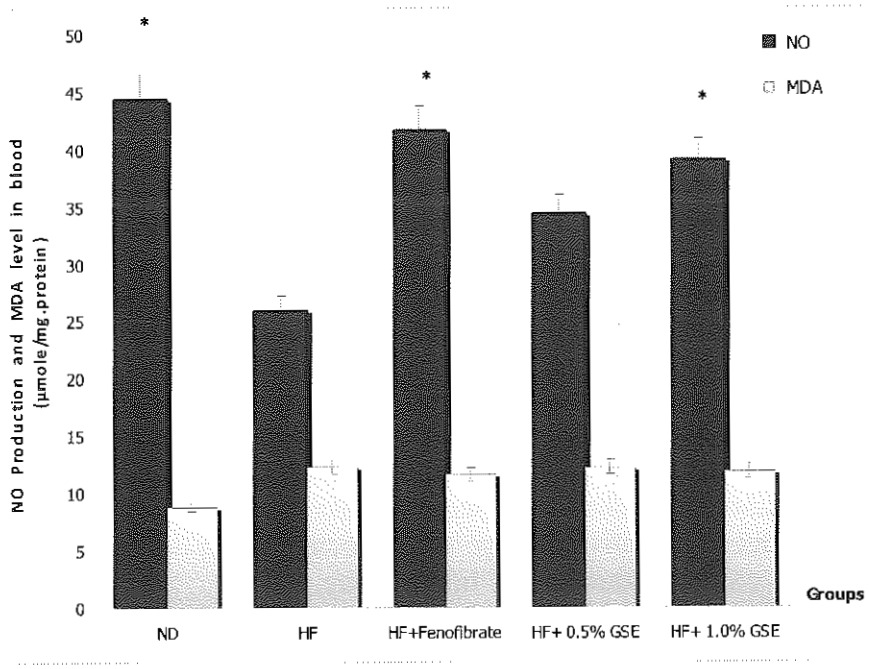

Figure 2 Effect of GSE (0.5% and 1%) on NO production and MDA levels in blood of rats for each group. Each value represents the mean ± SEM of 8 rats. * $p<$ 5 vs. HF-diet group.

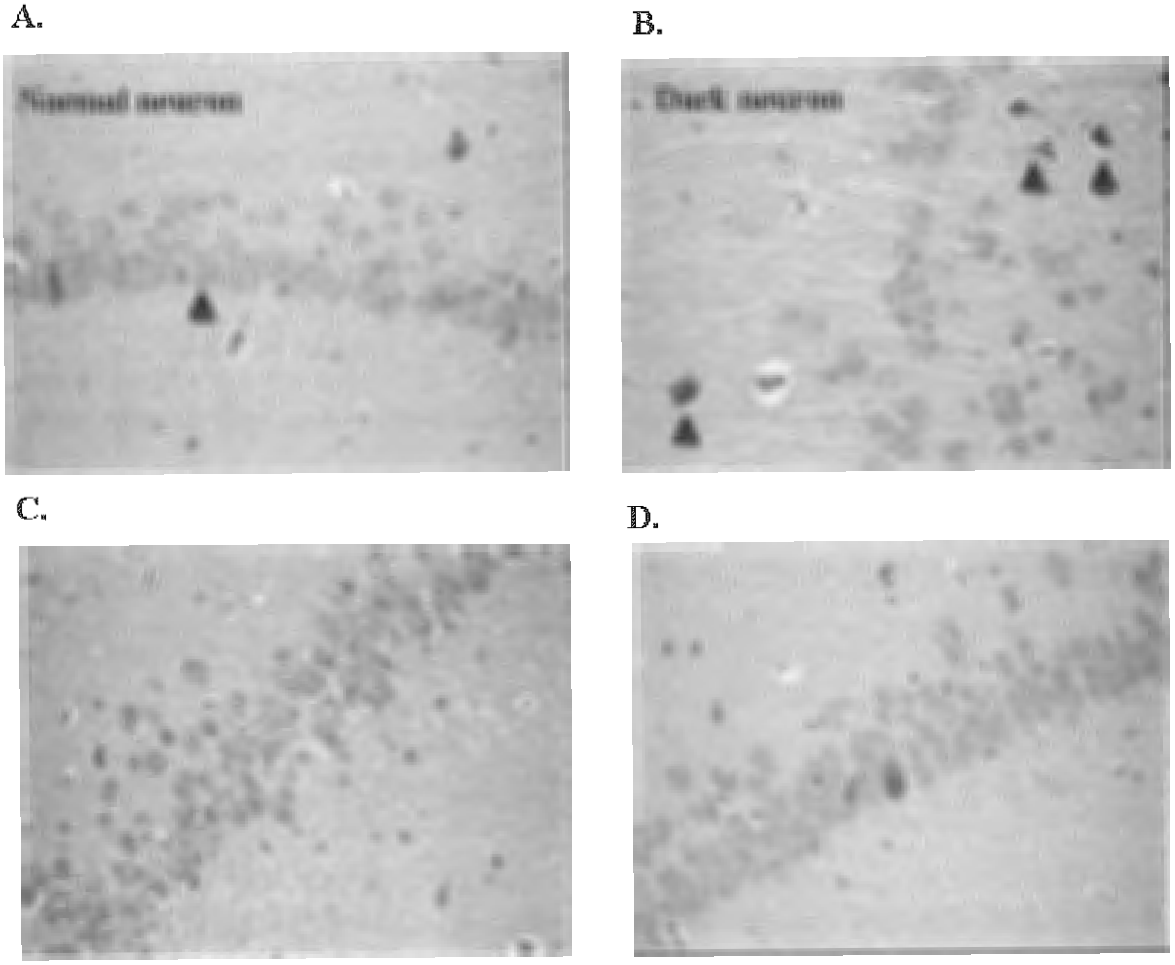

Figure 3 Row of nerve cells in a section of the pyramidal cell band of the hippocampus. (A) Normal, (B) HF-diet , (C) HF ± fenofibrate, (D) HF ± 1% GSE (40x, H&E stain).

Table 1 Distribution of histopathological scores for the occurrence of dark neurons

Groups	Brain regions		
	Cerebral cortex	Cerebellum	Hippocampus
ND	0	1+	0
HF	3+	2+	3+
HF+Fenofribrate	1+	2+	2+
HF+GSE 0.5%	1+	1+	2+
HF+GSE1.0%	1+	1+	1+

Disscussion and conclusion

High fat diet cause oxidative damage demonstrated by increasing the levels of NO and MDA in brain tissue. Oral treatment with lipid lowering drug (fenofibrate) and GSE can prevent lipid peroxidation and pathological changes in the brain tissue. The neuroprotective effect of GSE on high fat diet induced oxidative damage was associated with the decrease in the lipid peroxidation and nitric oxide production in brain tissue.

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Original article **P14**

Antiplatelet Activity of Deferiprone in Healthy Volunteers

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Abstract

Abnormalities of platelet functions which leading to pulmonary thromboembolism are commonly found in β-thalassemic patients with iron overload. Iron mediated free radical has been found to be involved in platelet aggregation pathway. Therefore, deferiprone, an orally active iron chelator, may exert antiplatelet activities in addition to iron mobilizing activity. In this study, the *ex vivo* antiplatelet activity of deferiprone was studied in 11 healthy normal volunteers receiving a single dose of 25 mg/kg. Platelet-rich plasma (PRP) was prepared from blood samples collected at predose (0) and, 60 and 360 minutes after deferiprone administration. The maximum aggregation of platelet was monitored by an aggregometer after activated by adenosine diphosphate (ADP) at concentrations of 4 and 8 μM. At 60 minutes after deferiprone administration, the serum concentration of deferiprone was 15.29±5.45 μg/ml. The maximum platelet aggregation induced by ADP at concentrations of 4 and 8 μM significantly decreased about 50% and 40%, respectively comparing with baseline. Serum concentration of deferiprone became 2.31±0.67 μg/ml at 360 minutes, and the maximum platelet aggregation was not significant difference from that of baseline. This is a first report demonstrate the *ex vivo* antiplatelet activity of deferiprone. Further consideration should be given to the development of iron chelators for antithrombotic therapy.

Keywords: platelet aggregation, deferiprone, adenosine diphosphate

Introduction

Beta-thalassemia/hemoglobin E (β-thal/Hb E) which is a double heterozygosity between β-thalassemia and Hb E, is prevalent in Thailand. It is a β-globin variant resulting from substitution of Glu->Lys at codon 26. The frequency of the β-thalassemia gene is 3-9% and that of the hemoglobin E gene is up to 50% in some provinces.

A study on autopsied cases of β-thal/Hb E revealed that 44% of the patients had pulmonary artery thrombosis and it was found mainly in splenectomized patients (1). Furthermore, 71% of splenectomized and 35% of nonsplenectomized patients with β-thal/Hb E patients showed increased circulating platelet aggregates (2). Moreover, increasing in the urinary excretion of urinary metabolites of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) has been reported (3). These reports suggested that thalassemic platelets are enhanced activation. The existence of platelet activation in thalassemia was further confirmed by flow cytometric studies, which showed the presence of an increased fraction of platelets carrying the activation markers CD62P (P selectin) and CD63 (4). Because of iron overload, some studies demonstrated that in the presence of iron platelets primed with AA or collagen exhibit enhanced aggregation (5). Moreover, Iron mediated free radical has been found to be involved in platelet aggregation pathway (6).

Deferiprone (2-dimethyl-3-hydroxypyrid-4-one, L1), bidentate hydroxypyridones, is an oral iron chelator used for the treatment of iron overload in thalassemic patients. *In vitro* models, deferiprone was also shown to function as an antioxidant (7).

The study of the effect of deferiprone (L1) on human platelet function demonstrated that deferiprone (L1) inhibit human platelet aggregation, thromboxane A_2 synthesis and lipoxygenase activity in healthy normal *in vitro* model (8). Thus, the present study aimed to study the effect of deferiprone (L1) on platelet function in healthy normal volunteers and we proposed that deferiprone (L1), an oral iron chelator may exert antiplatelets activities in addition to iron mobilizing activity.

Methods

Blood samples were taken from 11 healthy normal volunteers who denied taking any drugs, particularly antiplatelet drugs for 2 weeks, received a single dose of 25 mg/kg. Platelet-rich plasma (PRP) was prepared from blood samples collected at predose (0), and, 60 and 360 minutes after deferiprone administration. The maximum aggregation of platelet was monitored by an aggregometer after activated by adenosine diphosphate (ADP) at final concentrations of 4 and 8 μM. Results were evaluated and calculated as maximum aggregation.

Results

Before administration of L1, maximum platelet aggregation induced by ADP 4 and 8 μM was about 16% and 26%, respectively. Inhibitory effect of L1 was observed at 60 minutes after deferiprone administration. The serum concentration of deferiprone was 15.29±5.45 μg/ml and platelet aggregation induced by ADP 4 and 8 μM significantly decreased about 50% and 40%, respectively, comparing with baseline (time 0). This effect of L1 was diminished when drug was cleared from the circulation (time 360).

Table 1 Serum deferiprone level in normal volunteers

Time (min)	Concentration of L1 (μg/ml)	Maximum Platelet Aggregation (%)	
		ADP = 4 μM	ADP = 8 μM
0	0	16.53±12.41] *	26.27±10.81
60	15.29±5.45	8.52±8.46]	18.73±9.69
360	2.31±0.67	11.35±10.83	20.11±10.68

Mean ± SD (n = 11), * = $p < 0.05$ compared with time 0

Conclusion

Our results demonstrate the antiplatelet activity of deferiprone in normal volunteers. This activity was reversible and related with deferiprone concentration in serum.

Acknowledgement

We wish to thank the Thalassemia Research Center, the Thailand Research Fund, and the Government Pharmaceutical Organization (GPO) for their support.

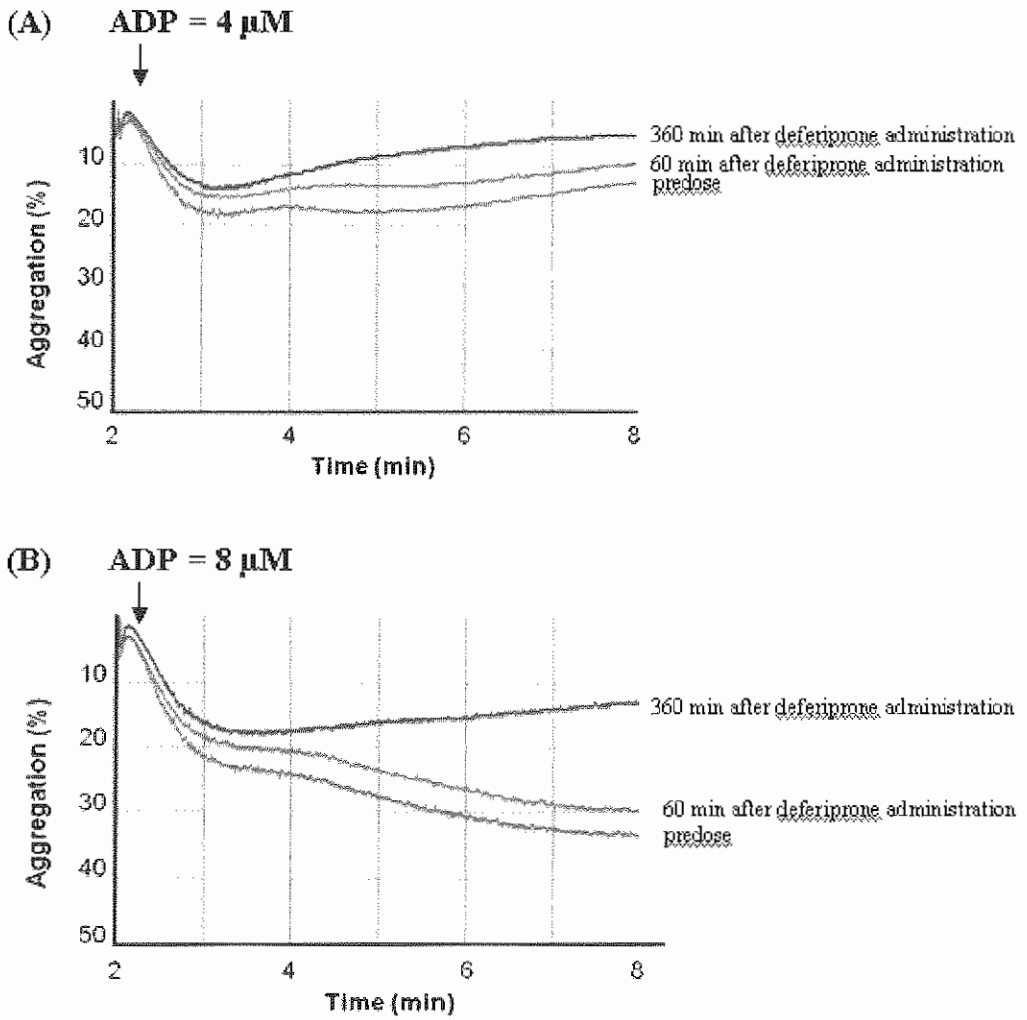

Figure 2 The platelet aggregation of PRP before and 60, 360 minutes after deferiprone administration: (A) ADP 4 µM, (B) ADP 8 µM.

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Original article **P15**

Prevalence of *CYP3A5* Polymorphism in a Thai Population

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Abstract

Human cytochrome P450 3A enzymes play an important role in the metabolism of numerous commonly used drugs. The CYP3A enzymes are the most abundant CYPs in human liver and small intestine. Substantial interindividual differences in CYP34 expression, exceeding 30 fold in some populations, contribute greatly to variation in oral bioavailability and systemic clearance of CYP3A substrates. Among CYP3A enzymes, the genetic polymorphism of *CYP3A5* has been extensively studied. Although ethnic difference in *CYP3A5* is well recognized, no data is available for this genetic polymorphism in a Thai population. Therefore, the aim of the study was to determine the *CYP3A5* polymorphism in a Thai population. The alleic frequency of the *CYP3A5*3* in 150 Thai healthy subjects was determined by real-time polymerase chain reaction using specific primers and TaqMan probes. The alleleic frequency of the *CYP3A5*3* in a Thai population was 66% and the genotype frequency of **1/*1*, **1/*3* and**3/*3* were 13.33%, 40.67% and 46%, respectively. The frequency of *CYP3A5*3* in a Thai population is similar to other Asian populations but different from that of Caucasians and African Americans.

Keywords: *CYP3A5*, Cytochrome P450, Thai population

Introduction

The CYP3A enzymes are the most abundant and important subfamily of cytochromes P450 in xenobiotic metabolism. They are responsible for the metabolism of 45%-60% of all currently used xenobiotics in human (1). The CYP3A locus of 231 kb located on chromosome 7q22.1 consists of *CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP3A43* genes and two pseudogenes *CYP34P1* and *CYP3AP2* (2). The CYP3A enzymes are involved in the oxidation, peroxidation, and reduction of approximately 50% of commonly used drugs, including cancer chemotherapeutic drugs, human immunodeficiency virus protease inhibitors, calcium channel antagonists, immunosuppressants, and cholesterol-lowering drugs (3). They are also involved in the metabolism of either carcinogens or toxic chemicals, as well as endogenous substances (4).

The interindividual variation in hepatic expression and activity of CYP3A isozymes is enormous and lead to pharmacokinetic variability in drugs that are CYP3A substrates. Several polymorphic CYP3A isoforms, particularly CYP3A4 and CYP3A5 have been recently described in different populations. Although CYP3A4 is the most abundant CYP isoform and the major contributor to CYP3A-mediated drug metabolism, genetic polymorphisms in CYP3A4 do not seem to be the cause of variability in drug disposition (5). Moreover, strong linkage disequilibrium has also been observed between the *CYP3A4* and

CYP3A5 SNPs. In a French population, 95% of patients homozygous for *CYP3A4*1* were homozygous for *CYP3A5*3*. They further hypothesized that ultimately *CYP3A5* genotype influenced the overall functional activity of CYP3As (1).

Among *CYP3A5* alleles, *CYP3A5*1* has been found to be the main allele associated with *CYP3A5* expression, whereas the mutant allele *CYP3A5*3* (SNP within intron 3, 6986G>A) prevents expression of the enzyme due to premature termination during translation of the aberrant mRNA (1) and causes alternative splicing and protein truncation resulting in the absence of CYP3A5 enzymes in tissues from some individuals. CYP3A5 may appear to constitute up to 50% of total hepatic CYP3A protein in individual carrying at least one *CYP3A5*1* allele. Thus, *CYP3A5*3* mutant allele may play an important role in interindividual and interethnic differences in the metabolic profile of many drugs (3). However, the alleic frequency of *CYP3A5* polymorphism in a Thai population has not been investigated.

Methods

Study population: Genomic DNA of 150 Thai healthy volunteers which left over from the Genetic polymorphism of *CYP2C19* in Thai populations project was used in this study. The use of these gDNAs was approved by the Ethic Committee on Human Research, Khon Kaen University (HE510851).

Genotyping: Genetic polymorphism of *CYP3A5* was determined by real-time polymerase chain reaction using specific primers and TaqMan probes.

Statistical Analysis: The 95% confidence intervals were calculated for all observed genotype frequencies. The Chi-square test was used for comparison of the allele frequencies between different populations. $P<0.05$ was considered significant. Statistics analysis was carried out by using the STATA package (version 8.2).

Results

Genotyping data from a total of 150 Thai healthy subjects were shown in Table 1.

Table 1. Genotype frequencies of *CYP3A5* in a Thai population

Genotype	Number of subject	Frequency (%)	95% CI
*1/*1	20	13.33	7.89-18.77
*1/*3	61	40.67	32.80-48.52
*3/*3	69	46.00	38.02-53.98
Total	150		

The observed genotype does not significantly deviate from the expected Hardy-Weinberg equilibrium ($\chi^2 = 3.57$, $P>0.05$). In this study, the allele frequency of *CYP3A5*1* in a Thai population were 34% whereas the allele frequency of *CYP3A5*3* allele was 66% and these values were significantly different from those observed in the Caucasians ($\chi^2 = 9.76$, $P=0.002$) and African Americans ($\chi^2 = 4.12$, $P=0.042$) but not statistically significant when compared to other Asian populations such as Chinese ($\chi^2 = 3.57$, $P=0.059$), Japanese ($\chi^2 = 2.97$, $P=0.085$), Indians ($\chi^2 = 1.04$, $P=0.307$) and Malays ($\chi^2 = 0.54$, $P=0.463$) populations (Table 2).

Table 2. Allelic frequencies of the *CYP3A5* in a Thai population and other ethnic populations

Ethnicity	Number of subject	% Alleic frequency		*P*-value
		*1	*3	
Thais (this study)	150	34	66	This study
Caucasians	27	15	85	0.002
African Americans	20	45	48	0.042
Asians				
Chineses	302	22	78	0.059
Japaneses	200	23	77	0.085
Indians	90	41	59	0.307
Malaysians	98	39	61	0.463

Discussions and conclusions

This is the first report of allele frequency of *CYP3A5* in a Thai population. The results of this study demonstrated that the frequency of *CYP3A5*3* in a Thai population is higher than Caucasian population. *CYP3A5*3* which is the genetic basic of CYP3A5 nonexpressor is thought to be an important genetic contributor to interindividual and interethnic variations in metabolism of CYP3A5 substrates such as tacrolimus, cyclosporine, diltiazem and amlodipine, etc. Therefore, the dosage regimen of drugs that metabolized by CYP3A5 may be difference between Thai and Caucasian population. Recently, the studies in Asian population have been shown that nearly half (51%) of their populations were CYP3A5 expressor carry at least 1 expressor allele, *1 (1). This observation is in contrast with the white population wherein 10% of population was CYP3A5 expressors (6). The consequence of this genetic polymorphism on dosage regimen of CYP3A5 substrate in a Thai population should be investigated further.

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Original Article **P16**

Comparative Pharmacokinetic Study of Two Single Doses of Deferiprone in β-thalassemia/Hb E Patients

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Abstract

Deferiprone (L1) is the first orally active iron chelator for treatment of blood transfusion dependent iron overload and thalassemia patients. Its efficacy and toxicity have been hypothesized that may relate to individual iron overload status and capacity of drug metabolizing enzyme. The pharmacokinetics of deferiprone and its conjugated metabolite (L1G) of two single oral doses, 25 and 50 mg/kg, were comparative studied in 12 β-thalassemia/Hb E patients. High performance liquid chromatography analysis of serum revealed that deferiprone was rapidly absorbed to circulation system and reached maximum concentration within one hour after administration. Pharmacokinetic parameters including C_{max} and $AUC_{0-\infty}$ of L1 and L1G were increased direct proportionally with the dose of deferiprone. A constant ratio of serum $AUC_{0-\infty}$ L1G to L1 and percentages of urinary excretion of L1G were observed, suggesting that the high dose did not excess the capacity of the deferiprone metabolizing enzyme (UGT1A6). Longer elimination half life ($elt_{1/2}$) of non-glucuronide deferiprone was observed in the high dose and significantly correlated with total serum chelated iron. These data suggest the possibility of deferiprone bound iron complex accumulation in the iron loaded cell consequence to delay of deferiprone excretion process.
Keywords: Deferiprone, Pharmacokinetics, β-thalassemia/Hb E

Introduction

Deferiprone (L1, 1, 2 dimethyl-3 hydroxypyrid α-4-one) is an orally active bidentate iron chelator that is developed for treatment of blood transfusion dependent iron overload in chronic anemias. Many clinical studies in patients with β-thalassemia have demonstrated that deferiprone 75 to 100 mg/kg/day can reduce iron burden in regularly transfused iron overloaded patients (1, 2). Unfortunately, there are still many inconvenient factors for using deferiprone as ideal iron chelator. Decreased trough plasma concentration of deferiprone during long term treatments is the main factor affecting to decrease iron excretion efficiency (3). Agranulocytosis, neutrophiles less than $0.5x10^9$/L, may has relationship with serum L1 concentration level due to dose-dependent bone marrow suppression effect (4).

Although many single dose and long term pharmacokinetic data of L1 are available, dose dependent pharmacokinetics was not available. Comparative pharmacokinetic study of two single doses in 12 severe β-thalassemia/Hb E patients in this study was evaluated in order to assist in understanding influence of deferiprone dosage to iron excretion efficiency. Finally, the results from this study may useful for optimize dosage regiment and improve therapeutic protocol for drug administration. Furthermore single dose pharmacokinetics of

deferiprone that is produced by Thailand Government Pharmaceutical Organization for Thai patients will also be first evaluated.

Materials and Methods

Twelve severe β-thalassemia/Hb E patients were enrolled in this study. At first period, patients received 25 mg/kg deferiprone after an overnight fasting. Blood samples were collected pre-dose, and 30, 60, 120, 180 and 360 min after dosing. Urine output was pooled and collected 0-6 and 6-24 hr intervals. Serum and urine concentrations of non-glucuronide deferiprone (L1 and L1-iron complex) and its glucuronide metabolite (L1G) were determined by HPLC. Pharmacokinetic parameters were calculated using noncompartment model. After two weeks of washout period, patients were given the second dose, 50 mg/kg, and the experiment was carried on with the same procedure.

Results

Pharmacokinetic parameters, maximum serum concentration (C_{max}) and area under concentration time curve ($AUC_{0-\infty}$), of L1 and L1G were increased direct proportionally with the dose of deferiprone (Figure1, Table 1). Interestingly, terminal elimination half life ($elt_{1/2}$) of L1 was also changed regarding to dose. It was significantly correlated with total serum chelated iron, r_s values was -0.832 ($p=0.001$) and 0.685 ($p=0.014$) in 25 and 50 mg/kg deferiprone dosage, respectively. However, ratio of $AUC_{0-\infty}$ of serum L1G to L1 was constant with 1.27±0.62 and 1.32±0.42 for 25 and 50 mg/kg administration, respectively. Urinary excretion of L1G was about 89% of total L1 excretion and there was no significant difference between the two doses as shown in Table 2.

Figure 1: Serum concentration-time profile of L1 (a) and L1G (b) in β-thalassemia/Hb E patients received deferiprone dose 25 and 50 mg/kg. Data are presented as mean±SD; **p<0.01 *p<0.05 comparing with β-thalassemia/Hb E patients receiving deferiprone 25 mg/kg.

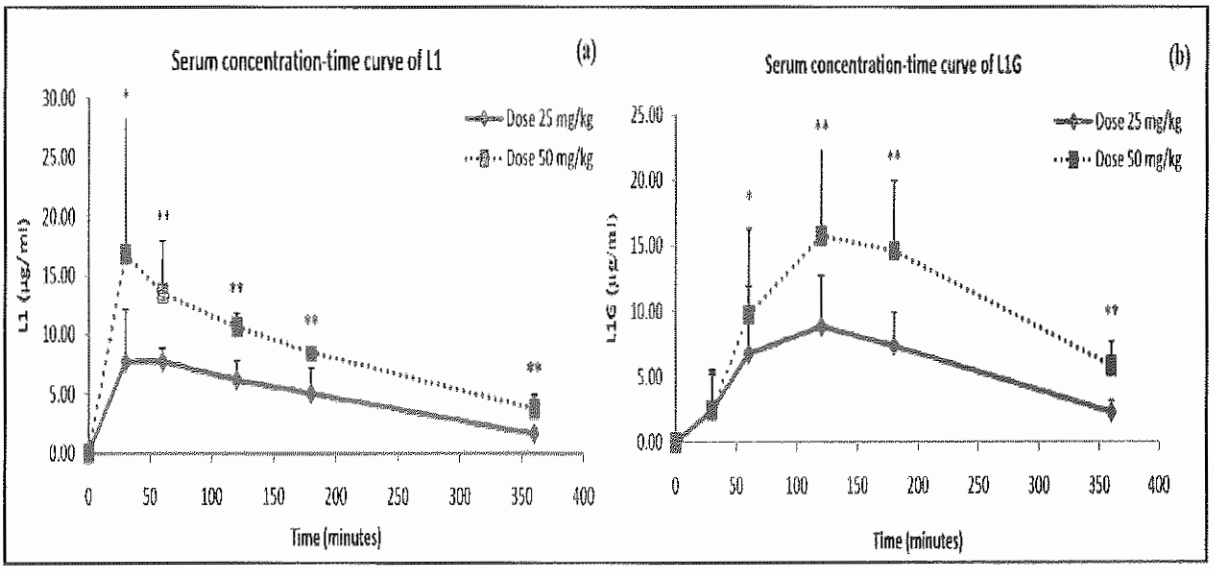

Table 1: Pharmacokinetic parameters of L1 and L1G in serum of β-thalassemia/Hb E

Deferiprone Dosage	*L1 PK*		*L1G PK*	
	25 mg/kg (n=12)	50 mg/kg (n=12)	25 mg/kg (n=12)	50 mg/kg (n=12)
C_{max} (μg/ml)	10.0 ± 2.3	19.7 ± 9.3 **	9.3 ± 4.4	16.1 ± 6.4 **
T_{max} (min)	45 (30-180)	60 (30-120)	120 (60-180)	120 (120-180)
$elt_{1/2}$ (min)	116.0 ± 16.0	155.6 ± 45.0 *	113.3 ± 34.1	178.4 ± 151.4*
$AUC_{0-\infty}$(μg.min/ml)	1,966.7 ± 378.6	4,019.2 ± 697.1 **	2,383.8 ± 824.4	5,335.0 ± 1819.4**
Vd/F (ml/kg)	2,212.2 ± 624.9	2,750.7 ± 560.7	-	-
CL/F (ml/min)	586.8 ± 178.6	563.1 ± 140.8	-	-

Data are presented as mean ± SD except for T_{max} [median (min-max)]; **p<0.01 *p<0.05 comparing with β-thalassemia/Hb E patients receiving deferiprone 25 mg/kg.

Table 2: Twenty four hrs urinary excretion of L1 and L1G in of β-thalassemia/Hb E

Parameter	*Deferiprone Dosage*	
	25 mg/kg (n=12)	50 mg/kg (n=12)
Deferiprone (mg)	70.59±22.41	125.82±53.48 **
(%administrated dose)	6.58±2.22	5.76±2.39
Deferiprone-glucuronide (mg)	626.87±208.59	1,191.61±492.42 **
(%administrated dose)	56.01±14.80	53.86±19.31
Percentage of L1 recovery	62.61±14.99	59.63±20.59

Data are presented as mean ± SD; **p<0.01 *p<0.05 comparing with β-thalassemia/Hb E patients receiving deferiprone 25 mg/kg.

Conclusion

Not only linear alterations in deferiprone and its metabolite pharmacokinetic parameters including C_{max}, $AUC_{0-\infty}$ but also constant $AUC_{0-\infty}$ ratio of serum L1G to L1 and percentages of urinary L1 recovery indicate that the increasing dose of deferiprone did not limit capacity of drug metabolizing enzymes. High deferiprone dose-response effect in longer elimination half life was possibly affected from deferiprone-iron complex accumulation and consequence to iron excretion efficiency.

Acknowledgements

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Original article **P17**

Pharmacokinetic and Bioequivalence study of a single 8-mg dose of Rosiglitazone tablets in Thai healthy volunteers

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Abstract

Rosiglitazone maleate is an antihyperglycemic agent in the thiazolidinedione class. A new product of rosiglitazone has been developed. The bioequivalent data of new generic product is required in order to assure the quality and performance. Pharmacokinetic parameters and bioequivalence of generic product of a single oral 8 mg rosiglitazone tablet with the innovator's product were studied. The study was performed in 24 healthy Thai male volunteers. Each received a single oral dose of 8 mg rosiglitazone tablet. Double blind randomized two-way crossover design was used with two weeks washout period between treatments. After drug administration, serial blood sample was collected over a period of 48 hours. Rosiglitazone plasma level was determined by HPLC with fluorescence detector. The pharmacokinetic parameters were determined by non compartment model. For bioequivalence determination, the difference of were analyzed by ANOVA and 90% confidence interval. The mean ± SD of pharmacokinetic parameters of generic product and the innovator's product were 0.82 ± 0.52 vs. 1.02 ± 1.50 hr of T_{max}, 796.51 ± 155.19 vs. 723.48 ± 134.69 ng/ml of C_{max}, 3.94 ± 0.80 vs. 3.87 ± 0.77 hr of $T_{½}$, 4308.43 ± 1,006.28 vs. 4135.66 ± 1061.96 ng.hr/ml of AUC_{0-t}, 4384.65 ± 1035.15 vs. 4183.87 ± 1075.39 ng.hr/ml of AUC_{0-inf}, respectively. The 90% confidence interval of mean difference of C_{max}, AUC_{0-t} and AUC_{0-inf} (log transformed data) of the innovator's product compared to generic product were 98.42-122.18%, 97.28-109.66% and 97.79-110.30%, respectively. They were within the range of the acceptance criteria 80 – 125%.

Keywords: Rosiglitazone, Pharmacokinetic, Bioequivalence

Original article **P18**

Chemopreventive Effect of Phenethyl Isothiocyanate in Cholangiocarcinoma Cells

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Abstract

Phenethyl isothiocyanate (PEITC), a natural compound found abundantly in cruciferous and other vegetables, has been shown to possess cancer chemopreventive activity. The purpose of this investigation was to examine the cytotoxic effect of PEITC in cholangiocarcinoma cells. Cholangiocarcinoma cells, KKU-100 and HeLa Chang cells which was derived from normal human liver were used for comparison in the study. Effects of PEITC on cell growth were determined by a sulforhodamine B assay and induction of apoptosis was determined by fluorescent dye staining using acridine orange and ethidium bromide. Cultured cells were exposed to PEITC for 48-hr following assays of cytotoxicity and apoptotic cell death. The cytotoxicity of PEITC on KKU-100 and HeLa Chang cells, expressed as IC_{50} were (mean ± SD) 7.22 ± 2.23 μM and 13.10 ± 1.23 μM, respectively. Moreover, PEITC can induce a large proportion of cells to undergo apoptosis in a concentration-dependent manner. It was concluded that the effect of PEITC on cell growth and apoptosis may contribute to the cancer chemopreventive properties.

Keywords. Phenylethyl isothiocyanate; Cholamgiocarcinoma; Apoptosis; Cytotoxicity

Acknowledgement: This work was supported by Khon Kaen University.

Original article **P19**

ESR Spin Labeling Studies on the Site of Iron-induced Free Radical Reaction in Low Density Lipoprotein

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Abstract

A possible mechanism to promote low density lipoprotein (LDL) oxidation, which believed to be crucial for the early event in atherogenesis, is free radical reaction. Kinetic and site of free radical reaction in the specific region, however, have not been exactly elucidated. In order to localize the specific site of LDL oxidation, paramagnetic fatty acids, 5- and 16-doxyl stearic acid (5- and 16-DS) were used to label phospholipid layer near hydrophilic surface and the deeper hydrophobic region of LDL, respectively. To induce free radical reaction in LDL isolated from healthy, normolipidemia male volunteers, either hemin or ferric nitrilotriacetate (Fe-NTA) was co-addition with either *tert*-butyl hydroperoxide (*t*-BuOOH) or hydrogen peroxide (H_2O_2). Decreasing of middle field ESR signal height was monitored versus time to calculate rate of ESR signal decay. Hemin, but not Fe-NTA, induced ESR signal decay. In the presence of 100 μM hemin coaddition with 1 mM H_2O_2, rate of ESR signal decay of 5- and 16-DS was 2.81±0.67 $\times 10^3$ and 8.08±2.85 $\times 10^3$ AU/min, respectively. Furthermore, the coaddition with 1 mM *t*-BuOOH, rate of ESR signal decay of 5- and 16-DS was 1.94±0.30 $\times 10^4$ and 3.54±1.72 $\times 10^4$ AU/min, respectively. Our results suggested that the rate of ESR signal decay could represent the rate of free radical reaction in the specific site of LDL which depended on species and concentration of iron and hydroperoxide. The deeper hydrophobic region of LDL is a primary site of LDL oxidation.

Keywords: LDL, Doxyl stearic acid, ESR spin labeling

Introduction

Free radicals are highly reactive species and may promote oxidation of proteins, peroxidation of membrane lipids, and modification of nucleic acids. An increase in the steady state levels of ROS beyond the antioxidant capacity of the organism, called oxidative stress, is encountered in many pathological conditions. Low density lipoprotein (LDL), a transport vehicle for water-insoluble lipid in the blood, is a highly compartmentalized system consisting of phospholipids, unesterified cholesterol (UC), and apolipoprotein (apoB-100) in the outer layer with cholesteryl esters (CE), triglyceride (TG), and antioxidants distributed in the core (1). The oxidation hypothesis proposes that LDL must be oxidative modification to become oxidized LDL (Ox-LDL) which believed to be crucial for the early event in the genesis of atherosclerotic lesion. Wide variety of mechanisms have been proposed for mediated LDL oxidation, including free and protein-bound metal ions (2). Recent studies investigated the iron-induced oxidative effects in LDL. These results suggested that iron-induced LDL oxidation at the hydrophobic region showed good correlation with oxidative

stress makers, such as the formation of thiobarbituric acid-reactive substances (TBARS) and conjugated diene (CD), ratio of cholesteryl linoleate (CL) to cholesteryl oleate (CO), (CL/CO), and lipid fluidity (3, 4, 5). A possible mechanism to promote LDL oxidation is free radical reaction, however, there was no directed evident showed that free radical reaction is initiated in core region of LDL. This present study designed to develop the technique of ESR spin labeling to localize the site of iron-induced free radical reaction in LDL.

Materials and methods

LDL was prepared from blood sample obtained from healthy, normolipidemia male volunteers. Blood samples were centrifuged at 2,330g at 4°C for 10 min to obtained serum. Pooled serum of volunteers were separated LDL by sequential density gradient ultracentrifugation, which is modified from Havel method. Paramagnetic fatty acids 5- and 16- doxyl stearic acid (5- and 16-DS), which dissolved in hexane to desired concentration and dry under nitrogen in clean glass tube, were used to label phospholipid near hydrophilic polar head group and the deeper hydrophobic region of LDL, respectively. Either hemin or ferric nitrilotriacetate (Fe-NTA) in various concentrations about 20 to 100 μM was added with either 1 mM *tert*-butyl hydroperoxide (*t*-BuOOH) or hydrogen peroxide (H_2O_2) to induce free radical reaction. Lipid mobility of LDL was determined by X-band ESR spectrometer with microwave frequency 9.8 GHz, power 10.13 mw, amplitude 100 kHz, and field modulation 0.25 and 0.125 for 5- and 16-DS, respectively. Decreasing of middle field ESR signal height was monitored versus time and the rate of ESR signal decay was calculated.

Results

Figure 1 shows ESR spectrum of 5- and 16-DS incorporated in LDL. Time course effect after incubation of hemin and Fe-NTA is showed in Figure 2. The addition of hydroperoxide and hemin, a lipophilic Fe^{3+}-containing protoporphyrin IX resulted in decreasing of ESR signal of both 5- and 16-DS. In contrast a water-soluble iron complex, Fe^{3+}-NTA, did not cause ESR signal decay.

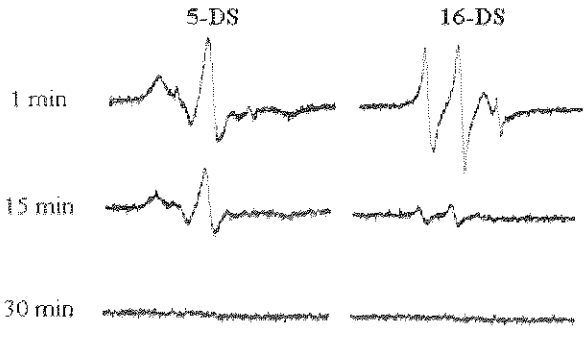

Figure 1. ESR spectrum of 5- and 16-DS in the present of hemin and peroxide.

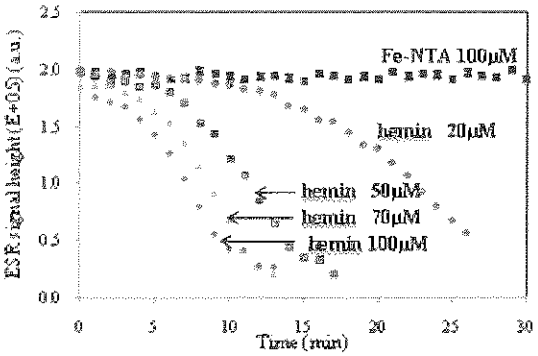

Figure 2. Time course of ESR signal decay in LDL induced by hemin and Fe-NTA coaddition with hydroperoxide.

Table 1. showed rate of ESR signal decay of 5- and 16-DS induced by hemin co-incubated with *t*- BuOOH or H_2O_2. With the same experimental condition, rate of ESR signal decay of 16-DS was faster than 5-DS. Moreover, *t*- BuOOH showed a higher potency than H_2O_2 to induce the signal decay of the both probe.

Table 1. Rate of ESR signal decay ($\times 10^4$ Au/min) of free radical reaction induced by hemin and hydroperoxide.

Hemin (μM)	5-DS		16-DS	
	t-BuOOH	H_2O_2	*t*-BuOOH	H_2O_2
20	0.84±0.30	0.07±0.06	1.49±0.57	0.49±0.29
50	1.66±0.40	0.24±0.05	2.63±1.20	0.70±0.41
70	1.77±0.44	0.27±0.08	4.14±2.09	0.80±0.36
100	1.94±0.30	0.29±0.07	3.54±1.72	0.81±0.29

Data are presented as mean ± SD of 3-independent experiments

Conclusion

Our results suggested the rate of ESR signal decay could represent the rate of free radical reaction in the specific site of LDL which depended on species and concentration of iron and hydroperoxide. It might suggest that oxidative modification of LDL via free radical reaction might initiate in the deeper hydrophobic domain.

Acknowledgement

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Original article **P20**

Association of Dyslipidemia with Coronary Artery Stenosis in Coronary Artery Disease Patients

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Abstract

Dyslipidemia is one of the major risk factors for coronary artery disease (CAD). It is evident that oxidative stress plays a significant role in the pathogenesis of coronary artery atherosclerosis and its complications. This study was conducted to assess the oxidant status and evaluate the association of dyslipidemia with the degree of coronary artery stenosis in thirty six patients undergoing coronary angiography. Body mass index, heart rate, blood pressure and coronary risk factors were investigated. Fasting venous blood was analyzed for lipids and plasma malondialdehyde (MDA). The significant elevated serum triglycerides (TG) and decreased serum high-density lipoprotein (HDL) cholesterol were presented in 23 patients with coronary stenosis (>50% in $\geq$ 1 vessel). The levels of TG and HDL-cholesterol were correlated with the number of stenotic vessels (r = 0.352, p = 0.035 and r = -0.351, p = 0.036, respectively). A slight increase in plasma MDA, a lipid peroxidation marker, was observed in CAD patients. These data suggest that the severity of CAD is associated with atherogenic dyslipidemia, and CAD patients are prone to the oxidant stress condition.

Keywords: Coronary artery disease, Dyslipidemia, Oxidative stress

Introduction

CAD is a leading cause of death and disability worldwide in both developed and developing countries. The development and progression of atherosclerotic heart disease comprises various processes, such as endothelial dysfunction, oxidative stress, chronic inflammation, and lipid profile modification(1). Elevated serum triglyceride (TG) and decreased HDL cholesterol levels are commonly found in many patients with established CAD(2). Atherogenic dyslipidemia (defined as high TG and low HDL cholesterol) has been indicated as the central component of the metabolic syndrome and plays a key role in development of CAD(3). Therefore, the purpose of this study was to evaluate whether the levels of lipid profiles is associated with the severity of coronary artery stenosis in patients with angiographically documented CAD.

Materials and methods

1. Study population and lipid peroxidation assay

The study was approved by the Khon Kaen University Ethics Committee for Human Research, and all participants provided written informed consent. A total of 36 patients who had been undergone coronary angiography for assessment of CAD at Queen Sirikit Heart

Center of the Northeast Hospital, Khon Kaen University, from August 2008 to January 2009 were recruited in this study. All participants were divided into two groups, the CAD and control groups. CAD was defined as a stenosis of ≥ 50 % detected angiographically in the major coronary vessel, whereas the non-CAD (control group) was diagnosed as no significant narrowing of the coronary arteries. The inclusion criteria were angiographically documented CAD and a stable condition. Each subject was screened by a complete history, physical examination, and laboratory analysis. Exclusion criteria included unstable angina, recent myocardial infarction, recent coronary angioplasty, valvular heart disease, evidence of heart failure, end stage renal disease, inflammatory disease and malignancy. The fasting blood samples were collected for routine hematological and biochemical assays, and the plasma was kept at -80 °C until the time of oxidant analysis. The assay of malondialdehyde (MDA), a lipid peroxidation marker, was performed in plasma as thiobarbituric acid reactive substances (TBARS) (4). After the reaction of MDA with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm.

2. Statistical analysis

Results are presented as mean ± S.E.M. Comparison between the two groups was used Student's *t*-test. Pearson correlation analysis was assessed for the relationships between variables. *P* value less than 0.05 was considered significant. Analyses were performed using the Stata version 7 (Stata Corp., College Station, TX, USA).

Results

Table 1 shows the baseline characteristics of patients with CAD and non CAD (controls). Average age among the 36 participants was 60.3±1.5 years. There were no significant differences in age, body mass index, heart rate, blood pressure, fasting blood glucose, total cholesterol, LDL-cholesterol and coronary risk factors between CAD and control groups. However, a significant elevation of TG and reduction in HDL levels was found in the CAD patients ($P<0.05$).

Table 1 Baseline characteristics of the study population.

	Controls (n=13)	CAD (n=23)
Gender, male/female	2/11	16/7
Age, year	58.1 ± 2.2	61.6 ± 2.0
Body mass index, kg/m^2	25.6 ± 0.9	25.7 ± 0.6
Heart rate, beats/min	64.1 ± 3.2	64.5 ± 2.1
Systolic blood pressure, mmHg	130.1 ± 4.1	128.2 ± 3.1
Diastolic blood pressure, mmHg	76.7 ± 2.2	76.0 ± 2.4
Lipid status		
Total cholesterol, mg/dL	195.9 ± 12.5	193.2 ± 9.5
Triglyceride, mg/dL	167.2 ± 16.6	235.4 ± 21.6*
HDL-cholesterol, mg/dL	46.9 ± 3.2	39.3 ± 1.9*
LDL-cholesterol, mg/dL	115.5 ±10.8	107.4 ± 8.4
Fasting blood glucose, mg/dL	91.4 ± 5.1	108 ± 8.9
Coronary risk factors		
Hypertension, *n*	7	14
Dyslipidemia, *n*	2	7
Diabetes, *n*	1	5
Current smoker, *n*	2	6

Values are mean ± S.E.M., n, number, *$P<0.05$ *vs.* controls.

The number of stenosis vessel was correlated with the levels of TG ($r = 0.352$, $p = 0.035$) and HDL-cholesterol ($r = -0.351$, $p = 0.036$), whereas other parameters showed no significant correlations (Table 2). Plasma MDA was slightly increased in CAD patients ($p = 0.057$, Fig. 1), indicating an enhancement of oxidative stress in these patients.

Table 2 The correlation between number of stenosis vessel and other parameter measurements in all subjects.

	r	*P* value
Age	0.181	0.292
BMI	0.073	0.671
Total cholesterol	-0.149	0.385
Triglyceride	0.352	0.035*
HDL-cholesterol	-0.351	0.036*
LDL-cholesterol	-0.243	0.152

*$P<0.05$ *vs.* controls

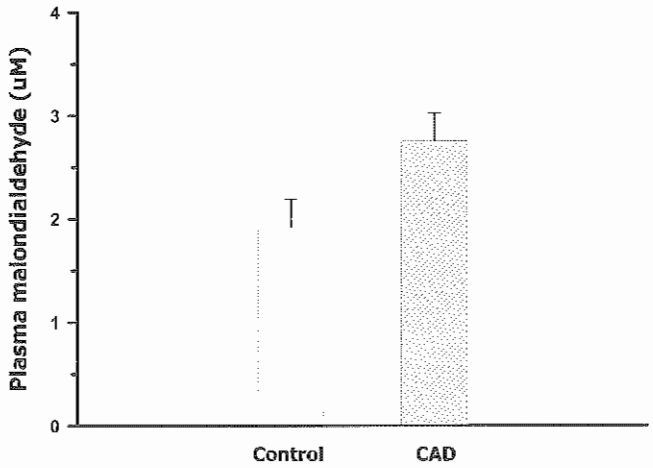

Figure. 1 Plasma malondialdehyde in control and CAD patients.

Discussion

In the present study, we demonstrated that dyslipidemia is a good predictor for the severity and the extent of coronary artery stenosis in patients with CAD. Previous study suggests that atherogenic dyslipidemia is a strong and independent predictor of cardiovascular risk outcomes in patients with angiographically documented CAD (3). Several potential mechanisms have been proposed to explain how TG may promote atherogenesis, including enhancement of oxidative stress, reduction of HDL-cholesterol, influence on LDL size distribution, induction of cell adhesion molecule expression and effects of TG-rich lipoproteins and fatty acids on the endothelium of vessel wall (5). Our study also found a tendency elevation of oxidative stress in CAD patients, suggesting the presence of an oxidant stress condition in CAD. In conclusion, our findings suggest that elevated TG, and low HDL-cholesterol levels may be used as a criterion to identify the severity of coronary artery

stenosis in patients with CAD. These predictors may be useful for the CAD patients with poorer prognoses in order to target for more specific therapeutic interventions.

Acknowledgements

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Original article **P21**

Effects of Asiaticoside and Madecassoside on Human Cytochrome P450

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Abstract

Introduction: Asiaticoside and madecassoside are the major triterpenoid compounds from *Centella asiatica* (Linn.) Urban. Asiaticoside was reported to possess wound healing activity, anti-inflammatory, protection of gastric ulcer and free radical scavenger. Madecassoside exhibited anti-inflammatory, antioxidant and rheumatoid arthritis healing activities.
Objective: The purpose of this study was to investigate the effects of asiaticoside and madecassoside on the activities of human cytochrome P450 (CYP) including CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 by using recombinant human CYP.
Materials and methods: Inhibition of CYP450 activities was determined by using the Vivid® CYP450 Screening Kits Protocol. The Vivid® Substrates were metabolized by specific CYP enzymes into products that were highly fluorescent in aqueous solutions.
Results: The results demonstrated that asiaticoside and madecassoside possessed inhibitory effects on CYP3A4 and CYP2C19 while the inhibitory effects of both compounds on CYP1A2, CYP2C9, CYP2D6 and CYP2E1 were not shown.
Discussion and Conclusion: The inhibitory effect of asiaticoside and madecassoside on CYP3A4 and CYP2C19 activities suggested that these compounds were needed to be further investigated whether they would cause drug-drug interaction with medicines that are metabolizes by these enzymes.

Keywords: Asiaticoside, Madecassoside, Cytochrome P450

Introduction

Asiaticoside and madecassoside are the major triterpenoid compounds from *Centella asiatica* (Linn.) Urban. *C.asiatica* is a pantropical plant in Thailand that is normally used as food, beverage and traditional medicine (1,2). There are many preclinical studies of asiaticoside and madecassoside for the pharmacological activity. Asiaticoside was reported to possess the effects of anticancer (3), wound healing activity, anti-inflammatory, protection of gastric ulcer and free radical scavenger (4). Madecassoside exhibited anti-inflammatory (5), antioxidant (6) and rheumatoid arthritis healing activity in mice (7).

During the research and development process of any new compounds, metabolism/ drug-drug interaction studies are required. Effect of any compounds on CYP is a beneficial information regarding drug-drug interaction and the possibility of the compound to increase/decrease risks of xenobiotic-induced toxicity/mutagenesis/carcinogenesis. In 2007, Seeka investigated the effect of the standard extract of *Centella asiatica* (ECa 233) on human

CYP by using recombinant human CYP (8). The results showed that ECa233 inhibited CYP2B6, CYP3A4 and CYP2C19 with IC_{50} less than 1,000 µg/ml. As asiaticoside and madecassoside are the major constituents in ECa233, the aim of this study was to investigate effects of both compounds on human CYPs by using recombinant human CYPs in an *in vitro* study.

Materials and Methods

1.Materials

Dimethylsulfoxide (DMSO) and Trizma® base were purchased from Sigma Chemical Co. Ltd., USA. Acetonitrile anhydrous were purchased from Labscan Asia CO. LTD, Thailand. Ethanol and hydrochloric acid (HCl) were purchased from Merck, Germany. Asiaticoside and madecassoside were kindly provided by Associate Professor Dr. Chamnan Patarapanich, Faculty of Pharmaceuical Sciences, Chulalongkorn University. The Vivid® CYP450 Screening Kit was purchased from Invitrogen Drug Discovery Solutions, USA. It consists of five components as following:

- CYP450 BACULOSOME® Reagents consisted of recombinant human cytochrome P450 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4) and rabbit NADPH P450 reductase
- Vivid® Substrates comprised either 7-benzyloxymethyloxy-3-cyanocoumarin (BOMCC) or ethoxymethyloxy-3-cyanocoumarin (EOMCC) depending upon the individual CYP isoforms.
- Vivid® CYP450 Reaction Buffers were potassium phosphate pH 8.0 in the different concentrations depending upon the individual CYP isoforms.
- Regeneration System consisted of Glucose-6-phosphate (G6P) and Glucose -6-phosphate dehydrogenase (G6PD) in potassium phosphate pH 8.0
- $NADP^+$ in potassium phosphate pH 8.0

2. Enzymes assay

Inhibitions of CYP activities were determined according to the Vivid® CYP450 Screening Kits Protocol (www.invitrogen.com). The Vivid® Substrates (BOMCC and EOMCC) were metabolized by a specific CYP enzyme into a product that was highly fluorescent in aqueous solutions.

Briefly, in 96 black well plate, add 40 µl of the compound solution per well and then add 50 µl of the mixture of CYP450 BACULOSOME® Reagents and Regeneration System in Vivid® CYP450 Reaction Buffers or Master Pre-mix. The mixtures were preincubated for 20 min at room temperature. Then the reaction was started by adding 10 µl of the mixture of reconstituted substrate and $NADP^+$ in Vivid® CYP450 Reaction Buffers and incubated for 30-60 min at room temperature. At the end of incubation, 10 µl of 0.5 M Tris-HCl buffer, pH 10.5 was added to quench the reaction. The fluorescence of product was measured by Fluorescence, Absorbance and Luminescence Reader VICTOR3V (Perkin Elmer, USA).

3. Statistical analysis.

Percent inhibition was calculated for each concentration of the test compounds by using the following equation.

$$\% \text{ inhibition} = \left[\frac{1 - (RFU_{\text{test compound}} - RFU_{\text{background of test compound}})}{(RFU_{\text{solvent control}} - RFU_{\text{background of solvent control}})} \right] \times 100$$

RFU – relative fluorescence unit or fluorescence intensity

The median inhibition concentration (IC_{50}) was calculated for the test compounds by using Probit analysis of SPSS 16.0 software.

Results

The results showed that asiaticoside inhibited activities of CYP3A4 and CYP2C19 with IC_{50} of 343.35 μM and 412.68 μM, respectively. Madecassoside inhibited activities of CYP3A4 and CYP2C19 with IC_{50} of 388.11 μM and 539.04 μM, respectively. However, no effects of asiaticoside and madecassoside on CYP1A2, CYP2C9, CYP2D6 and CYP2E1 were observed (Table 1).

Table 1: IC_{50} values of asiaticoside and madecassoside on human CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 activities

	IC_{50} values (μM)					
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Asiaticoside	>1000	>1000	412.68 (331.43-502.90)	>1000	>200*	343.35 (236.75-562.67)
Madecassoside	>1000	>1000	539.04 (467.40-624.86)	>1000	>1000	388.11 (286.50-526.08)

Data presented IC_{50} values with 95% confidence interval from four experiments (n=4).

* The data was limited by the solubility of asiaticoside and the amount of DMSO.

Discussion and Conclusion

Inhibitory effects of asiaticoside and madecassoside on CYP3A4 and CYP2C19 activities suggested the possibility of these compounds regarding drug-drug interaction if these compounds were administrated concomitantly with other medicines that are metabolizes by these enzymes. Further study should be investigated whether this inhibitory effect would be clinically significant.

Acknowledgements

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Original article **P22**

The Anti-arthritis Activity of Phlai + Ginger Gel in Adjuvant-induced Arthritis Rats

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Abstract

Chronic arthritis has a high prevalence in elderly people. The rhizome of *Zingiber montanum* (Koenig) Link ex Dietr. or called in Thai "Phlai" has been found an anti-inflammatory activity. Phlai+ginger gel was a preparation formulated by Pharmaceutical and Natural Products Department, Thailand Institute of Scientific and Technological Research (TISTR). The objective of this study was to assess the anti-arthritis activity of this preparation. Adjuvant-induced arthritis model was conducted in rats. Data showed that Phlai+ginger gel significantly reduced percent inflammation of the right knee joint and decrease some of joint pathological changes when compared to the gel base group. In conclusion, Phlai+ginger gel have some anti-arthritis activity and may be used as an alterative drug for topical-arthritis therapy.

Keywords: Anti-arthritis, *Zingiber montanum*, Adjuvant-induced arthritis model

Introduction

Chronic arthritis is the inflammatory joint disease and represents higher prevalence in elderly people. More than 80 % of 60-year old people are threatening with this disease that causing pain and disability. There are mainly three forms of chronic arthritis, osteoarthritis, rheumatoid arthritis and gout. Osteoarthritis is a degenerative of articular cartilage while rheumatoid arthritis is an autoimmune disease affecting to joint synovial membrane. And gout is a metabolic joint problem. All of the above are ultimately complicated with joint inflammation. The inflammation of joint which recruits cell infiltration to the joint cavity, articular cartilage, synovial membrane will establish a pathological changes such as synovial hyperplasia, bone erosion, narrowing of the joint and ankylosis. The rhizome of *Zingiber montanum* (Koenig) Link ex Dietr. (In Thai, Phlai) has been recognized as an topical anti-inflammation in Thai traditional medicine for a long time. One of its active compound, (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) possesses a potent anti-inflammatory activity through the inhibition of cycloxygeneous (CO) and lipoxygeneous (LO) pathways and seems to have more prominent effects on the LO pathway (1). There are many extracted Phlai preparations in the market claimed relieving pain or anti-inflammatory activity but non of those represents the anti-arthritis activity. Our study aimed to assess the potency of Phlai+ginger gel on anti-arthritis activity by topical application. So the animal model was employed for studying the local effect of drug and Complete Freund's adjuvant was chosen to induce inflammatory mono-arthritis in rat.

Materials and methods

Animals

Female Sprague Dawley rats (250 ± 20 g) were obtained from National Laboratory Animal Centre ,Mahidol University, Salaya, Nakornpathom. They were kept in cages with wood shavings as bedding at 24 ± 2°C in 12 h light/dark cycle and feed with standard diets and tap water ad libitum. The animals were acclimatized for 7 days prior to the experiments.

Drugs

Phlai+ginger gel was formulated by Thailand Institute of Science and Technological Research (TISTR). The active compounds are a mixture of Phlai and ginger extracts in 4% w/v concentration. Diclofenac is one of the available commercial preparations in market containing 1% diclofenac.

Methods

The thirty-two rats were used and randomly allocated into 4 groups. Each group contained 8 animals and were treated with the following: group 1 (sham):no treatment ; group 2 (control):gel base 300mg/each ; group 3:Phlai+ginger gel 300 mg/each and group 4: Diclofenac gel 100 mg /each. The animals were anesthetized by using xylazine hydrochloride prior to inject with 0.2 ml of CFA into the right knee joint. A couple of CFA injections were performed with one week interval. After the second CFA injection, each treatment was applied topically one time for 7 consecutive days. The exception of sham group received 0.9% sodium chloride injection with no treatment. The right joint diameter was measured once a day by Mitutoyo verniacaliper for calculated in term of percent inflammation. All animal were euthanized by carbon dioxide asphyxia. The right hind limbs, including the knee and tarsal joints, were fixed and preserved in 10% formalin. After decalcification, the tissues were embedded in paraffin, sectioned at 5-μm thickness and stained with hematoxylin and eosin (H&E). The H&E preparations were microscopically observed focusing on the pathological changes of knee joint.

Percent inflammation = (A-B) X 100/B

A = Knee joint diameter (cm) after the second intraarticular injection
B = Knee joint diameter (cm) before second intraarticular injection

Results

After the 2nd CFA injection, the right knee joint dramatically swelled in all groups excepted sham group. Given 0.9% sodium chloride into joint cavity resulted a slight swelling. Thus, the intraarticular injection did not a harmful procedure to produce a joint swelling. Rats received gel base produced the highest percent inflammation along day 2-day 6. The inflammatory reaction phase peaked on day 3. The gel base group still maintained joint swelling until the end of experiment while the Phlai+ginger gel group showed significantly decrease of percent inflammation on day 4,5,6 ($p < 0.05$) when compared to the gel base group. The diclofenac group obviously inhibited joint swelling also. The histopathological examination was done to confirm a clinical finding. Sham group showed normal finding. Inflammatory cell infiltration (monocyte and polymorphonuclear cell) in cancellous bone, articular cartilage destruction, circumarticular fibrosis, exudates in joint cavity and synovial membrane hyperplasia were found in animals treated with gel base only. Animals applied

Phlai+ginger gel showed a reduction in all of the item compared with gel base group, while those applied diclofenac showed only cell infiltration in cancellous bone.

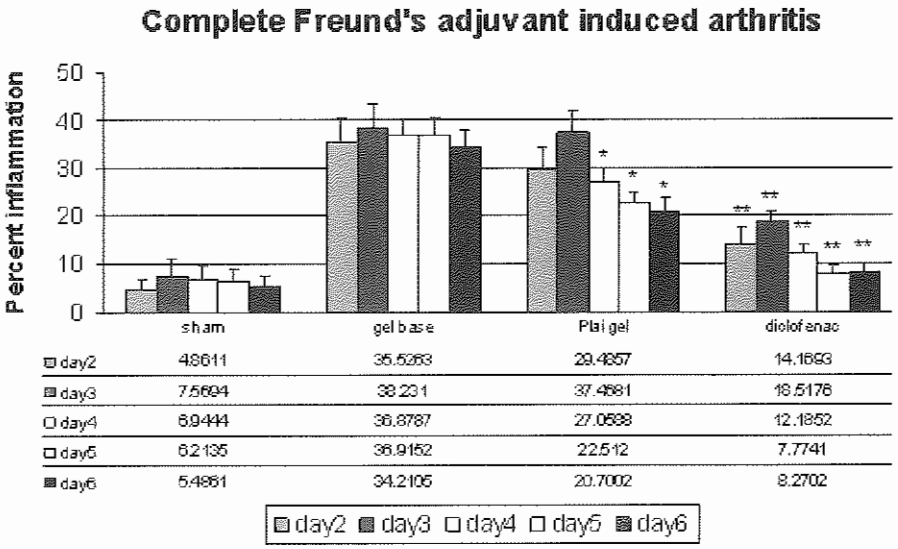

Figure 1 Anti-inflammatory activity of Phlai+ginger gel on arthritis
Inflammation of right knee joint was presented in term of percent inflammation. Graph shows mean ± SE of 8 animals from day 2 to day 6. The statistical significance of the differences were assessed by Student's T test.
*, ** showed significant differences at $p < 0.05$ and $p < 0.01$ respectively when compared to the gel base group.

Discussion

There are various methods to induce arthritis in animals. For those using carrageenan and kaolin are implied to acute arthritis model while subcutaneous injection of CFA will mediate chronic polyarthritis that seems like rheumatoid arthritis disease in human (2). This present study intended to evaluate the potency of drug as local effect, so we modified the chronic inflammatory monoarthritis by using a couple IA injection of CFA. After induction, only injected knee joint produced swelling while the contralateral joint remained unchanged (data not show).

Prostaglandins are mediator for acute inflammation but chronic inflammation are mediated by proinflammatory cytokine such as TNF-α.. The articular cartilage destruction, circumarticular fibrosis, and ankylosis are the pathological changes found in chronic inflammation. Phlai+ginger gel can reduce some level of this changes perhaps play an action through TNF-α . In conclusion, Phlai+ginger gel showed some benefit on chronic arthritis both in clinical and pathological aspect. The future study may investigate a mechanism of action.

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Original article **P23**

Bioavailability of a combined tablet formulation containing Lamivudine / Stavudine compared with each reference formulation concurrently administered in healthy Thai male volunteers.

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Abstract

This study evaluated the bioavailability of a combined tablet formulation containing lamivudine 150 mg and stavudine 30 mg compared with each reference formulation. A randomized, two way, crossover study was conducted in 26 fasting healthy volunteers. Blood samples were collected throughout a 24-hr period after administration of one combined tablet / or one tablet of each two reference drug. The plasma lamivudine / stavudine concentration were determined via HPLC-UV technique. Bioequivalence between the products was determined by calculating 90% confidence interval (90%CI) for the ratios of C_{max}, AUC_{0-t} and $AUC_{0-infinity}$. values for the test and reference products, using logarithmic transformed data. The result for lamivudine, the 90% CI of C_{max} (97.51-113.51%), AUC_{0-t} (95.44-105.75%) and $AUC_{0-infinity}$ (95.18-104.90%) values for the test and reference products were within the criteria of acceptance. And for stavudine, the 90% CI of C_{max} (96.62-118.13%), AUC_{0-t} (98.78-103.32%) and $AUC_{0-infinity}$ (98.21-104.07%) values for the test and reference products were also within the criteria of acceptance, proposed by Thai FDA. Two formulation were considered bioequivalent in the rate and extent of absorption.

Keywords : Bioavailability, lamivudine, stavudine

Original article P24

Wound Healing Effects of a Standardized Extract of *Centella asiatica* ECa 233 on Burn Wound in Rats

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Abstract

The present study aimed to investigate the effects of a standardized extract of *Centella asiatica* ECa 233 on second degree burn wound in Male Wistar rats. Gel containing 0.05% of ECa 233 as well as a gel base was topically applied, once daily, to a wound generated on the back side of the animals by a 90°C hot plate. Evaluation of wound healing including a visual observation, an estimation of rate of wound healing and measurement of cutaneous blood flow was made at day 3 and 7 post-burning. In general wound treated with ECa 233 was seemed to heal better than those of untreated or gel base-treated groups. Rate of wound healing in rats treated with 0.05% ECa 233 gel was significantly higher than those of untreated and gel base treated groups in day 7 post-burning. In agreement with the healing rate, cutaneous blood flow on day 3, both gel base and 0.05% ECa 233 groups were increased, whereas on day 7 only in 0.05% ECa 233 gel group was increased. Thus, it is likely that increases of oxygen and nutrient brought about by an increment of cutaneous blood flow may, at least, in part, be responsible for the wound healing effects of ECa 233. However, other mechanisms that are relevant to wound healing effects of ECa 233 remain unexplored. Therefore, it is suggested that studies on effects of ECa 233 on burn wound should be further carried out.

Keywords: *Centella asiatica*, second degree burn, wound healing

Introduction

Thermal burn is more commonly induced in tissues by the sudden exposure to excessive thermal energy. The local and systemic inflammatory responses to thermal burn are extremely complex, resulting in both local burn tissue damage and deleterious systemic effects on all other organ systems distant from the burn area itself. These include fluid and protein losses, local and systemic sepsis, gross metabolic, hematological and immune disturbances (1). These result in morbidity and mortality associated with burn injury (2,3). Burn wound care is needed according to the severity of burn. The concepts of optimum minor burn wound treatment focus on avoiding wound infection and treat with topical antibiotic agents or ointment, occlusive and wet dressing (3). Topical antibiotics are used routinely in the forms of antimicrobial creams e.g. silver sulfadiazine applied locally to skin injury.

Despite the existence of many advanced medical treatments, we still have to encounter the side effects and high expense. In Thailand, there are many kinds of herbs including *Centella asiatica* (Linn.) that have been advocated for their wound healing effect.

Madecassoside, one of the major constituent of *C. asiatica* has significant wound healing activity in parallel with a decrease of nitric oxide (NO) levels and malondialdehyde (MDA) content in the burn skin tissue when administered orally at higher doses (12 and 24

mg/kg) (4). Therefore, we decided to investigate the wound healing effects of ECa 233, which is a white to off-white of standardized extracted powder of *C.asiatica* containing triterpenoids not less than 80% and the ratio between madecassoside and asiaticoside was kept at 1.5±0.5, on burn wound in rats.

Materials and methods

1.Animals

Male wistar rats weighing 250-300 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. The rats were caged in the air-conditioned room maintained temperature at 25±1°c. They were provided with food and water *ad libitum* and acclimatized for 1 week before experimentation. Rats were divided into four groups as follows: no burn, burn with no treatment (untreated), burn treated with gel base, burn treated with 0.05 % ECa 233 gel.

2. Induction of second degree of burn injury

The animals were anesthetized with pentobarbital sodium 60 mg/kg intraperitoneally. Second-degree burn wound was made by placing the 90°c hot plate on the selected area of the back of the animal for 10 sec (5). The wounds were topically treated with 100 mg of gel base or gel base containing 0.05% ECa 233 once daily.

3. Evaluation of burn wound

Rate of wound healing

On day 3 and 7 post burning, photographs of the wounds were taken by digital camera. The areas of wound were measured by Image Tool v.3.0 and rate of wound healing was calculated using the following formula (4).

wound healing rate (%) = [(wound area on day 0 - wound area on day N) / wound area on day 0] x 100%

Cutaneous blood flow

Measurement of cutaneous blood flow was carried out by a Laser Doppler Flowmeter (Perimed AB SE-175, Periflux System 5000) (6). The needle probe was fixed perpendicularly on skin. Five different measurements (at the center and four corners) were performed and the mean was used for calculation as percent change compared to normal rats.

4. Statistical analysis

Results are presented as mean ± S.E.M. The differences among experimental groups were compared by one-way ANOVA followed by Duncan post hoc test and were considered statistically when P was less than 0.05.

Results

General appearance of the wound

On day 3 post-burning, wound in untreated and gel base-treated groups became swelling and exudated. In comparison, the wound in 0.05% ECa 233 gel group showed a mild degree of swelling and wound surface was rather dry and smooth. On day 7 post-burning, most of wounds treated with 0.05% ECa 233 gel showed apparent wound contraction, becoming smaller in diameter when compared to the untreated and gel base-treated groups. In addition, hair formation was also noted in ECa 233 treated group.

Effect of ECa 233 on rate of wound healing

No significant effects on rate of wound healing were observed on day 3. The rate of wound healing was found to be 14.12±2.48, 16.12±2.64 and 21.23±1.89 % in the untreated,

gel base-treated and 0.05% ECa 233-treated groups, respectively. On day 7 post-burning, rate of wound healing in 0.05% ECa 233-treated group (42.52±1.88%) was significantly higher than untreated and gel base groups (Figure 1).

Effect of ECa 233 on cutaneous blood flow

In comparison to unwounded skin, blood flow was found to be increased in all groups of animals with wound. On day 3 post-burning, cutaneous blood flow of gel base and 0.05% ECa 233 treated groups (144.44±10.75% and 166.35±12.90 %, respectively) were significantly higher than untreated group (109.59±8.21%). However, on day 7, cutaneous blood flow of only the 0.05% ECa 233-treated group (148.14 ± 8.77) but not the gel base-treated group (134.66 ± 9.65%) was significantly different from untreated group (Figure 2).

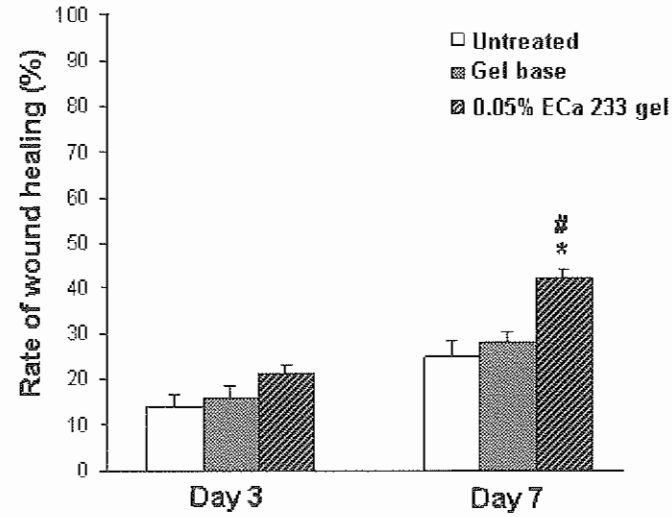

Figure 1 Rate of wound healing in different treatment groups on day 3 and 7 post burning
Values are mean ± S.E.M. (n=6). * Significantly different from untreated group ($p < 0.05$).
Significantly different from gel base group ($p < 0.05$)

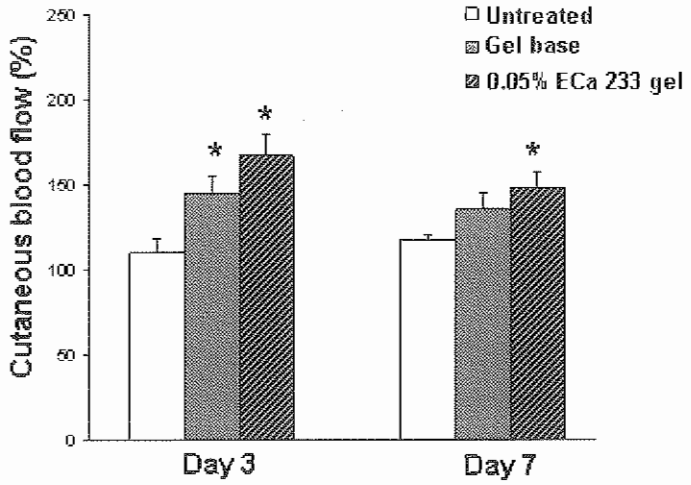

Figure 2 Relative cutaneous blood flow (percent changed from unwound skin) in treatment groups on day 3 and 7 post burning. Values are means ± S.E.M. (n=6).
* Significantly different from untreated group ($p < 0.05$)

Discussion and Conclusion

In burn wound, microcirculation is generally compromised to the worst extent at around 12-24 h post-burning resulting in a restriction or cessation of blood flow which in turn causes ischemia and subsequently a reperfusion and thus a generation of oxidative stress. Intervention that could restore blood flow or scavenging the free radicals should, in principle reduce the damage due to oxidative stress (7,8). The present study demonstrated the wound healing effect of topically applied standardized extract of *Centella asiatica* (ECa 233) on the burn wound model in rats. On day 3 post-burning, despite an increase in cutaneous blood flow in all groups, the 0.05% ECa 233 gel group was seemed to heal better than the other groups as contraction of wound edge was noted. The wound surface was rather dry and showed very mild degree of swelling, whereas exudates were apparent in untreated and gel base treated group.

On day 7, the cutaneous blood flow in untreated and gel base treated was rather similar and significant increase of blood flow was exclusively maintained in 0.05% ECa 233-treated group. In line with the observation on cutaneous blood flow, rate of wound healing in 0.05% ECa 233 treated-groups was significantly increased in comparison to gel base treated and untreated groups. Increase in cutaneous blood flow by ECa 233 should provide adequate perfusion and subsequently oxygen and nutrient essential for wound healing process resulting in a higher rate of wound healing observed. However, some other effects/mechanisms such as free radical scarvenging activity of ECa 233 that might be relevant to different phases of wound healing should be further conducted.

In conclusion topical application of standardized extract of *Centella asiatica* (ECa 233) clearly facilitated burn wound healing in rats. Though increase of blood flow is a plausible mechanism underlying the wound healing observed, some other mechanism should be further investigated.

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Original article **P25**

Anti-inflammatory Effect of the Phlai Oil, Distilled from 1 Year Old *Zingiber* montanum. (Koenig) Link ex. Dietr. Rhizome

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Abstract

The Phlai oil, obtained by hydrodistillation from 1 year old *Zingiber* montanum. (Koenig) Link ex. Dietr. rhizome was used to evaluate to the anti-inflammatory activity of croton oil induced rat ears edema model. Phlai oil at the dose 20 mg/ear reduced the rat ears edema formation by 27.8% (2hr), 45.4% (3 hr) and 52.6% (4hr) respectively *cf.* the control. The Phlai oil showed a potent anti-inflammatory effect.

Keywords: Phlai oil, Zingiber montanum. (Koenig) Link ex. Dietr. rhizome t, Anti-inflammatory activity

Introduction

According to the tradition knowledge, the 3 year old *Zingiber* montanum. (Koenig) Link ex. Dietr. (Syn: *Zingiber cassumunar* (Roxb.) rhizome and the essential oil, extracted from the rhizomes were recognized and previously used to scientifically investigate the biological effects. It shows potent anti-inflammatory, antimicrobial analgesic and etc. In this study, the anti-inflammatory effect of Phlai oil, extracted from 1 year old *Zingiber* Zingiber montanum. (Koenig) Link ex. Dietr. rhizome was conducted, using croton oil induced rat ear edema model.

Materials and methods

1. Phlai oil

The Phlai oil was obtained by hydrodistillation from 1 year old Zingiber montanum. (Koenig) Link ex. Dietr. rhizome

2. Animal

Male Wistar rats of body weight between 120-150 g were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. The animals were housed in animal care facility at Thailand Institute of Scientific and Technological Research for 1 week before experimentation.

3. Methods

Croton oil-induced rat ear edema model

Male Wistar rats were divided into groups of six animals. Rat ears were measured by pocket thickness gage before and after the croton oil were applied. The croton oil was applied the inner and outer surfaces of each rat ear for 2 hrs before and after the application to the rat ears at dose 20 mg/ear of Phlai oil (treated group) and acetone (control group). The ear thickness was measured 1, 2, 3 and 4 hr after test samples application.

Result and discussion

The anti-inflammatory effect of Phlai oil on croton oil induced rat ears edema was measured by pocket thickness gage. The essential oil, extracted from the 1 year old of *Zingiber montanum*. (Koenig) Link ex. Dietr. rhizome significantly showed a potent anti-inflammatory effect in rat ear edema model, compared to control (Fig. 1).

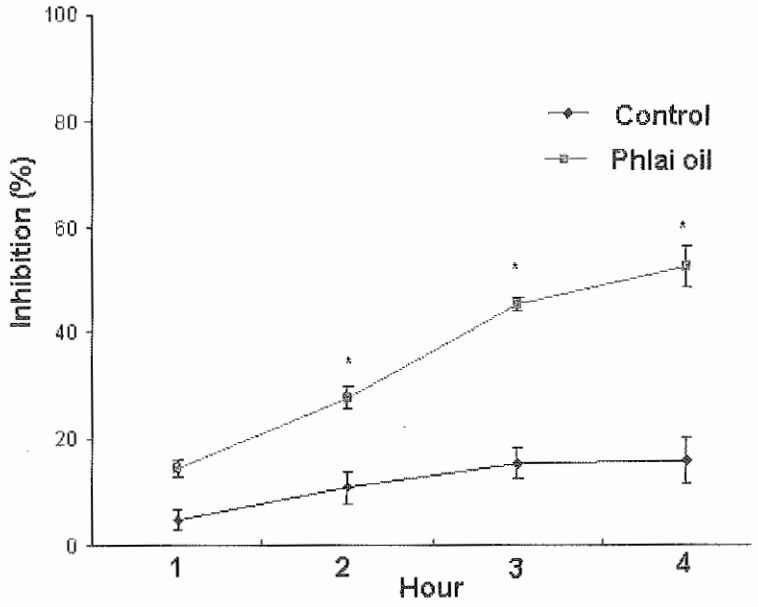

Figure1. Show the anti-inflammatory effect of Phlai oil, extracted from the 1 year old of *Zingiber montanum*. (Koenig) Link ex. Dietr. rhizome on croton oil induced rat ear edema. Percentage of edema thickness presented as mean ± S.E.

* Significantly different from control group ($p<0.05$)

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Original article **P26**

The Effect of *Phyllanthus emblica* on Hemin Induced Lipoprotein Oxidation

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Abstract

Lipoprotein oxidation is a key early stage in the development of atherosclerosis. Hemin (Iron (III)-protoporphyrin IX) can be detected in plasma of thalassemia patients. It is a potent oxidative inducer of lipoprotein oxidation. Dietary antioxidants can be used to protect lipoprotein oxidation. Ma-kham-pom (*Phyllanthus emblica* Linn) has been reported to have antioxidant activities. The aim of this study was to determine the protective effect of *P. emblica* on hemin induced lipoprotein oxidation. The pre-incubation of low-density lipoproteins (LDL) with various concentration of *P.emblica* (0.5, 1, 2.5, 5, 10 and 20 μg/mL) were performed; and then oxidized by hemin 5 μM/300 μg LDL protein (he-oxLDL) for 24 hr. L-ascorbic acid was used as a positive control. Lipoprotein oxidation was determined by measuring thiobarbituric acid reactive substances (TBARs) formation, a marker of lipid peroxidation, and platelet-activating factor acetylhydrolase (PAF-AH) activity, a lipid hydroperoxides scavenging enzyme. The results showed that *P.emblica* 20 μg/mL inhibited TBARs formation almost 100%. The 50% inhibition concentration (IC_{50}) of TBARs were 2.5 and 13 μg/mL for *P.emblica* and L-ascorbic acid, respectively. The PAF-AH activity in he-oxLDL incubated with 2.5-20 μg/mL of *P.emblica* were significant higher than he-oxLDL ($p<0.01$). We concluded that *P.emblica* can inhibit lipid peroxidation in he-oxLDL and preserved PAF-AH activity in a dose dependent manner.

Keywords: Lipoprotein oxidation, Hemin, *Phyllanthus emblica*

Introduction

Lipoprotein oxidation plays a role in the pathogenesis of atherosclerosis. There is increasing evidence that oxidation of plasma low density lipoprotein (LDL) is a critical factor in promoting atherosclerosis (1). Hemin (Iron (III)-protoporphyrin IX) is a degradation product of hemoglobin. Hemin was found to be elevated in pathological cases like severe hemoglobinopathies, sickle cell anemia and thalassemia (2). Several *in vitro* studies have revealed that hemin is an effective inducer of LDL oxidation. Therefore, the inhibition of LDL oxidation by supplementation of antioxidants becomes an attractive therapeutic strategy to prevent and possibly to treat atherosclerosis. *Phyllanthus emblica* Linn is commonly known as amla and names "Ma Kham Pom" in Thailand. It is a member of the family Euphorbiaceae. *P.emblica* has been shown to possess several pharmacologic actions and antioxidants. However, its effects on lipoprotein oxidation induced by hemin have not been established. Therefore, the aim of study was to determine the effect of *Phyllanthus emblica* on hemin induced lipoprotein oxidation by monitoring thiobarbituric acid reactive substances

(TBARs) formation, a marker of lipid peroxidation, and platelet-activating factor acetylhydrolase (PAF-AH) activity, a lipid hydroperoxides scavenging enzyme.

Methods

1. Oxidation of Lipoprotein The separation of LDL was performed by the sequential density gradient ultracentrifugation method. The powder of spray-dried from fruit juice of *P.emblica* was used in this study. LDL was pre-incubated with *P.emblica* at concentrations 0.5, 1, 2.5, 5, 10, 20 μg/mL for 30 minutes. Then hemin was added into LDL to induce lipoprotein oxidation (final concentration 5 μM hemin/300 μg LDL protein). L-ascorbic acid was used as a positive control. After incubation at 37 °C for 24 hr, the oxidation reaction was terminated by adding 50 μM of BHT. Then TBARs formation and PAF-AH activity were determined.

2. Thiobarbituric acid reactive substances The lipid peroxidation products were determined by spectrofluorometric method, with excitation and emission wavelength at 515 and 553 nm, respectively (3). 1,1,3,3-Tetraethoxypropane was used as a standard.

3. Platelet activating factor acetylhydrolase activity assay PAF-AH activity was measured by using 2-thio PAF substrate in 0.1 M Tris-HCl buffer (pH 7.2) containing 1 mM EDTA and 1 mM DTNB. Upon hydrolysis of the acetyl thio-ester bond at *sn*-2 position by PAF-AH, free thiols are detected using 5,5'-dithio-*bis*-(2-nitrobenzoic acid) (DTNB), the increase in absorbance at 405 nm was recorded continuously for 10 minutes (4).

4. Statistical analysis Data were expressed as mean±S.E.M. Differences between groups were tested with one-way ANOVA and correlation analyses were assessed by pearson correlation using the SPSS version 13.0 for window software. Statistically significant differences were accepted at $P<0.05$.

Results

P.emblica inhibited the TBARs formation in a dose dependent manner (Fig 1A). The maximum percent inhibitions of TBARs were 99.35% and 82.45% for *P.emblica* and L-ascorbic acid (20 μg/mL), respectively (Fig 2A.). The 50 percent inhibition concentration (IC_{50}) of *P.emblica* and L-ascorbic acid on TBARs formation was obtained from the plot between the concentrations against the percent inhibition of TBARs formation (Fig. 2A). The IC_{50} were 2.5 and 13 μg/mL for *P.emblica* and L- ascorbic acid, respectively. The PAF-AH activities in he-oxLDL incubated with *P.emblica* at 5, 10 and 20 μg/mL and with L-ascorbic acid at 20 μg/mL were significantly higher than he-oxLDL control (25.50±1.50, 26.8±1.90, 28.0±1.6 and 25.7±1.5 vs. 15.9±1.5 nmol/min/mg LDL protein, respectively, $p<0.001$) (Fig 1C and 1D). These results indicated that *P.emblica* was more effective than L-ascorbic acid to inhibit TBARs formation and to preserve PAF-AH activity. Significant inverse relationships were found between TBARs formation and PAF-AH activity with the r-value of -0.70 ($p<0.001$) as shown in the Figure 2B. While PAF-AH activity was depleted, the TBARs levels were increased.

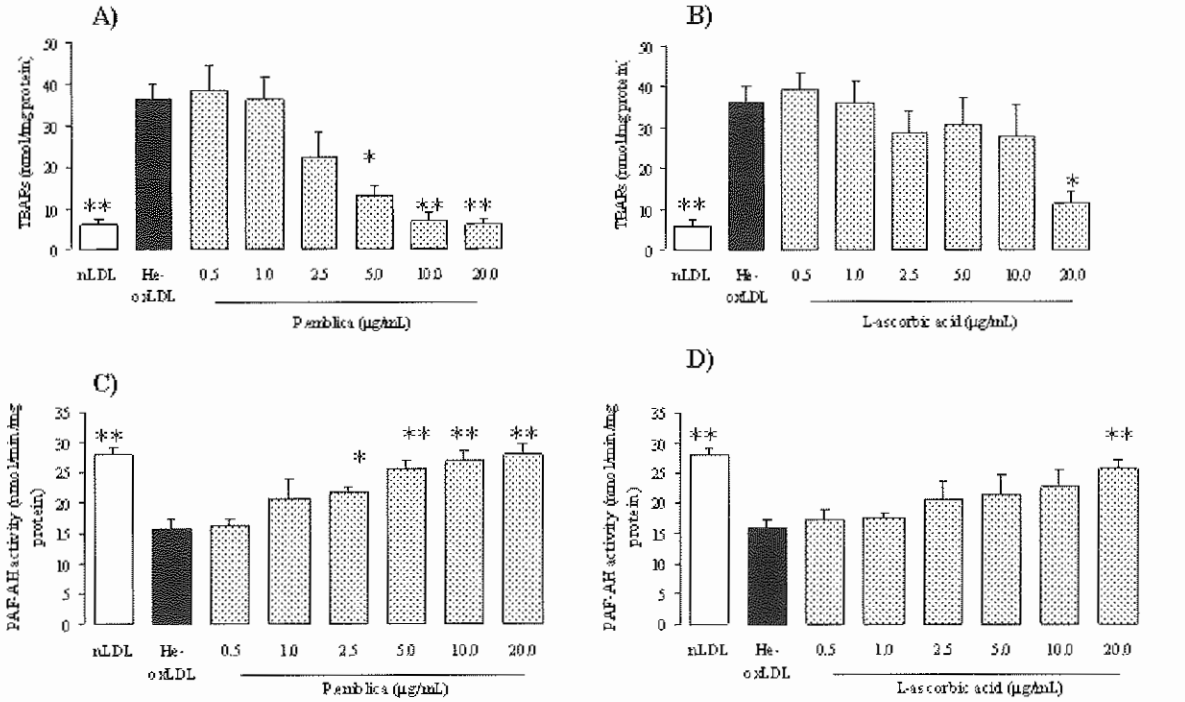

Figure 1 Effects of *P.emblica* and L-ascorbic acid on TBARs formation (A-B) and PAF-AH activity (C-D). Data are shown as mean±S.E.M. obtained from five independent experiments. * $p<0.01$ compared with He-oxLDL control, ** $p<0.001$ compare with He-oxLDL control

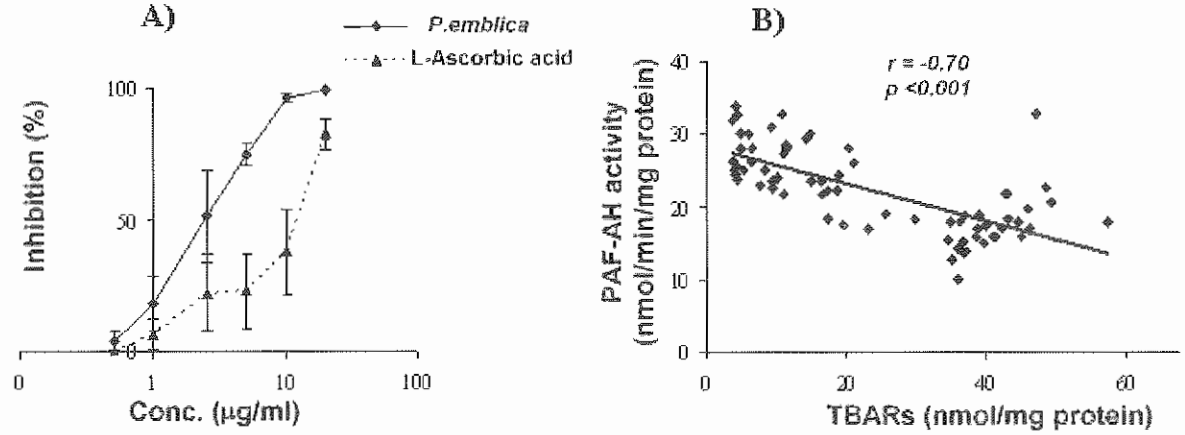

Figure 2 The percent inhibition of *P.emblica* and L-ascorbic acid on TBARs formation. The lines were acquired by plotting the concentrations of tested compounds (0.5, 1, 2.5, 5, 10 and 20 µg/mL) against the percent inhibition of TBARs formation (A). The correlation between TBARs with PAF-AH activity (B). The data were analysed from five independent experiments.

Discussion & Conclusion

P.emblica was successfully protected TBARs formation and preserved the PAF-AH activity. These effects possess the antioxidant activities. Since *P.emblica* contain with ascorbic acid and many phenolic compounds which have the potential to function as antioxidants. *P.emblica* has also been reported as a very effective free-radical scavenger (5). Our finding indicated that *P.emblica* was more potent to protect he-oxLDL than L-ascorbic acid. So we suggested that the inhibition of LDL oxidation of *P.emblica* may not be attributed to ascorbic acid alone but the overall effect is due to other polyphenols such as

ellagic acid, gallic acid, tannin, etc (6). In addition, the two new hydrolysable tannins, called emblicanin A and B are active constituents of *P.emblica* discovered, and have been reported to be a very strong antioxidant (7). Further study is needed to clarify the active substances and comparison of the potency of each compound in *P.emblica* extracts on the effect of hemin induced lipoprotein oxidation.

Acknowledgements

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Original article P27

Roselle Extract Enhances Glucose Uptake of Fat Cells Isolated from High Fructose and Fat-Diet Rats.

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Abstract

The diet high in fructose and fat can cause insulin resistance, impaired glucose tolerance and hyperinsulinemia. These metabolic changes have been implicated as contributing factors to the development of type 2 diabetes. Recently, we have found that roselle (*Hibicus sabdariffa*) extract has an antihyperglycemic activity in streptozotozin-induced type 1 diabetic rats. To investigate the antidiabetic efficacy of roselle extract for type 2 diabetes, this study examined the effect of water extract of roselle calyxes (HS-WE) on glucose uptake of adipocytes isolated from high fructose and fat (HFF) diet-induced impaired glucose tolerance rats. Male Sprague-Dawley rats were induced to have impaired glucose tolerance by feeding with HFF-diet for 12 weeks. Then, the oral glucose tolerance test was performed, and the basal serum insulin level was measured. The HFF-diet rats were sacrified and the epididymal fat pad was removed to prepare 40% fat cell suspension. The fat cell suspensions were treated with HS-WE (1-45 μg/ml) or insulin 1.5 nM. 2-deoxy-D-[U-^{14}C] glucose was then added and used as a tracer to measure the amount of glucose uptake into fat cells. We found that the 8-12 weeks HFF-diet rats had hyperinsulinemia and impaired glucose tolerance. HS-WE at 1, 5 , 15 and 45 μg/ml significantly increased glucose uptake into adipocytes as compared to basal glucose uptake by 120±3, 137±4, 148±4 and 126±3 %, respectively. In conclusion, our results indicated that the roselle extract may have glucose lowering activity by enhancing glucose uptake in HFF-diet rats.

Keywords: *Hibicus sabdariffa*, Insulin resistance, Impaired glucose tolerance

Introduction

In humans, the consumption of high-calories diets and sedentary life styles are the known causes of metabolic syndrome. In animals, diet high in fructose and fat causes multiple symptoms of metabolic syndrome such as insulin resistance, impaired glucose tolerance, hyperinsulinemia and hypertriglyceridemia (1) which are implicated as contributing factors in the development of type 2 diabetes. Nowadays herbal medicines are getting accepted throughout the world for treating diabetes. *Hibiscus sabdariffa* Linn (roselle), locally called "Krachiap Daeng" in Thailand, belongs to the Family Malvaceae. The extract of roselle calyxes has been reported of antidyslipidemia, antiatherosclerosis and antihypertensive activities (2). Recently, we have demonstrated that roselle extract has antihyperglycemia in streptozotozin-induced type 1 diabetic rats (3). However,

streptozotocin-induced diabetes does not imitate pathophysiology of type 2 diabetes which is the majority of diabetic patients. To investigate the antidiabetic efficacy of roselle extract for type 2 diabetes, therefore, this study had an aim to examine the effect of roselle water extract on glucose uptake of adipocytes isolated from high fructose and fat-diet rats.

Methods

1. Plant material and extraction: The dried calyxes of *H. sabdariffa* were blended and boiled with water (1:20) at 60°C for 60 minutes, and then filtered. The filtrate was concentrated using steam jacket boiler under vacuum, and was dried using Spray Dry machine. By this procedure the yield of the water extract of *H. sabdariffa* (HS-WE) was 14.2%.

2. The experimental animals: Male Sprague-Dawley rats (80-120 g) were obtained from the Animal Unit, Faculty of Medicine, Khon Kaen University. The animals were divided into two groups, Group I: feeding with normal diet and Group II: feeding with high fructose and fat (HFF)-diet. The HFF-diet consisted of 60% fructose, 20% coconut oil and 0.2% cholesterol in standard rat chow (C.P. Thailand) with drinking water containing 10% fructose *ad libitum*. All procedures were complied with the standards for the care and use of experimental animals and approved by Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand.

3. The oral glucose tolerance test (OGTT): At 8 and 12 weeks of feeding, the OGTT was performed. The animals were fasted for 14 hrs, then the animals were loaded orally with glucose 2 g/kg BW. The blood glucose before (0 min), at 30 and 120 min after glucose loading were determined using glucometer (Accu-check Advantage II).

4. Determination of serum insulin: At 8 and 12 weeks of feeding, the animals were fasted for 14 hrs and tail vein blood was collected to determine serum insulin levels using Rat insulin ELISA Kit (Linco, USA).

5. Determination of HS-WE effect on glucose uptake into adipocytes

Preparation of isolated fat cells: White adipocytes were prepared from the epididymal fat pad of HFF-diet rats by the method of Rodbell (4). Briefly described, rats were sacrificed by cervical dislocation, epididymal fat pad was removed and cut into pieces and digested by incubating with collagenase type I. The obtained cell suspension was filtered through nylon mesh and washed 3 times by floatation with KRBB without glucose to make a 40% packed cell volume.

Measurement of glucose uptake into fat cells **(5):** Cell suspension was transferred to microtubes and incubated for 15 min at 37°C. 2-deoxy-D-[U-^{14}C] glucose and insulin or HS-WE (final concentration: 1, 5, 15 and 45 µg/ml) were then added to those aliquots of cell suspension. All the treatments were done in triplicate. After incubation for 15 min at 37°C, the reaction was terminated by adding cytochalasin B. The fat cells were washed with cold KRBB for five times, by filtering through glass microfiber filters (Whatman®, U.K.) using high speed filter (Hoeter Scientific Instrument, USA.). The filter papers were placed in scintillation vials filled with aqueous scintillation cocktail. The radioactivity was counted by Beckman LS6500 Liquid Scintillation Counter (Beckman Instrument, U.K.).

6. Statistical analysis: Results were presented as mean±S.E.M. The blood glucose and serum insulin levels were compared between normal-diet and HFF-diet rats by unpaired *t*-test. The effect of HS-WE on the adipocyte's glucose uptake was evaluated using Analysis of Variance (ANOVA). A $\rho < 0.05$ is considered as statistical significance.

Results

The glucose tolerance of HFF-diet rats. The fasting blood glucose of HFF-diet rats and of normal-diet rats were not significantly different (Table 1). However, the % increase of blood glucose of HFF-diet rats at 30 and 60 min after glucose loading were significantly higher than that of normal-diet rats. This indicated that the HFF-diet rats had impaired glucose tolerance.

The serum insulin level of HFF-diet rats. The basal insulin levels (fasting state) of rats fed with HFF-diet for 8 and 12 weeks were significantly higher than that of normal-diet rats (Table 1). This indicated the hyperinsulinemia in HFF-diet rats.

Effect of HS-WE on glucose uptake. HS-WE at concentration of 1, 5, 15 and 45 μg/ml significantly increased 2-deoxy-D-[U-14C] glucose uptake into adipocytes isolated from HFF-diet rats in a dose-dependent manner (1,077 ± 25, 1,198 ± 28, 1,326 ± 21 and 1,143 ± 27 dpm, respectively) as compared to basal glucose uptake (968 ± 14 dpm.). In adipose cells treated with 1.5 nM insulin, glucose uptake (1,548 ± 11 dpm) was also significantly higher than that of basal glucose uptake (Fig. 1).

Table 1. Effect of high fructose and fat diets on oral glucose tolerance test and basal serum insulin

Groups	Basal insulin (ng/ml)	FBG (mg/dl)	% increase blood glucose	
			30 min	120 min
Normal-diet rats (n=5)	0.51±0.05	81.2±2.8	51.72	1.72
HFF-diet rats 8 weeks (n=7)	1.05±0.10*	68.3±2.3	85.57*	34.31*
HFF-diet rats 12 weeks (n=6)	1.16±0.09*	65.3±6.3	75.54*	43.75*

Values are mean ± S.E.M., n: number of animals, FBG: fasting blood glucose
*: $p<0.05$ as compared to control group

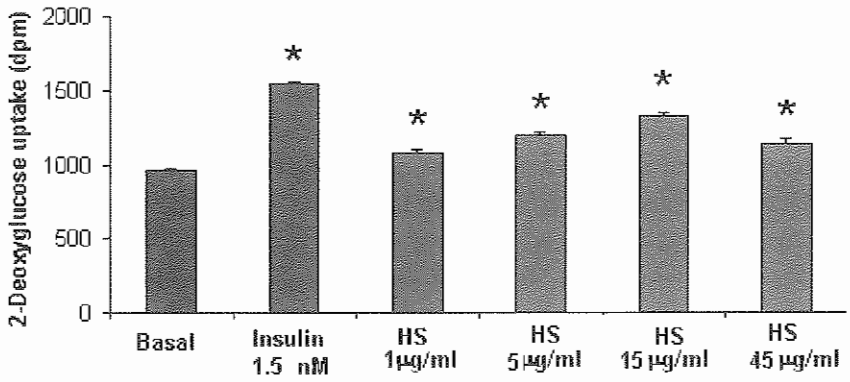

Figure 1. Effect of HS-WE on glucose uptake in adipocytes isolated from HFF-diet rats.
Values are mean ± S.E.M., *: $p<0.05$ as compared to basal uptake

Discussion

Feeding rats with high fructose and fat diet, is proposed to synergize their metabolic consequences; an approach that rendered rats to be insulin resistant with compensatory hyperinsulinemia, a condition similar to prediabetic, insulin resistant state in humans (6). Our experiment also demonstrated the insulin resistance in high fructose and fat diet rats as indicated by the impaired OGTT and hyperinsulinemia (Table 1). Fructose serves as an unregulated source of both glycerol-3-phosphate and acetyl-CoA for hepatic lipogenesis resulting in hypertriacylglyerolemia and finally, the free fatty acid-induced insulin resistance (1).

We found that HS-WE at the concentration of 1-45 μg/ml significantly increased the ^{14}C-glucose uptake of HFF-diet rat's adipocytes. This indicated that the HS-WE may be useful in the treatment of type 2 diabetic patients who are in insulin resistant state. However, the glucose uptake enhancing activity of HS-WE at the concentration of 45.0 μg/ml seemed to be smaller than the lower dose (15 μg/ml), this probably due the interfering effects of various substances containing in the crude extract of HS-WE, which may increase with the higher HS-WE concentration.

In conclusion, our results demonstrated that the roselle water extract may have blood glucose lowering activity by enhancing glucose uptake in high fructose and fat-diet rats.

Acknowledgement

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Original article **P28**

Crude Powder of *Curcuma comosa* Roxb Attenuates the Progression of Atherosclerosis in Cholesterol-fed Rabbits.

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Abstract

Curcuma comosa Roxb. is a well-known herb in Thailand and has been shown to reduce plasma cholesterol and triglyceride. This study aimed to investigate the effects of crude powder of *C. comosa* on the regression of preexisting atheromatous lesions and endothelial dysfunction in atherosclerotic rabbits. Forty-four male New Zealand White rabbits were randomly divided into 6 groups. Rabbits in the first two groups were fed normal rabbit chow for 4 or 16 weeks, served as the control-4- and -16-weeks groups. The other 4 groups were fed a diet containing 1% cholesterol for the first 4 weeks. One of these groups (cholesterol-4-weeks group) was studied after 4 weeks. The other 3 groups were followed for the next 12 weeks by 0.5% cholesterol-containing diet (cholesterol-16-weeks group), 0.5% cholesterol-containing diet with 5 mg of simvastatin (simvastatin group), 0.5% cholesterol-containing diet with 400 mg/kg/day of crude powder of *Curcuma comosa* (comosa group). Blood samples were collected for measurements of plasma lipid profiles. At the end of the experiments, endothelium-dependent and -independent vascular relaxations to Ach and SNP, respectively, and the total plaque areas in the left common carotid arteries were investigated. Treatments with 5 mg/day simvastatin or 400 mg/kg/day *C. comosa* decreased plasma lipids profiles, inhibited the progression of carotid intimal plaques, and reversed endothelium-dependent vasodilator function. Our present study shows that like lipid-lowering drug, crude powder of *C. comosa* improves NO-dependent vasodilator function and blocks the progression of plaques in pre-existing hypercholesterolemic rabbits. Its mechanisms need to be further investigated.

Keywords: *Curcuma comosa*, Endothelial function, Atherosclerosis

Introduction

Atherosclerosis is a disease of the vessel wall involving lipid accumulation, chronic inflammation, cell death, and thrombosis. Elevated plasma cholesterol is closely related to the extent of atherosclerotic lesions and the impairment of endothelial function (1-2). Dysfunctional endothelium plays a crucial role in all stages of atherosclerosis (3). One of the hallmarks of a dysfunctional endothelium is diminished levels of bioavailable nitric oxide (NO) (4-5).

Curcuma comosa Roxb. is a well-known herb belonging to Zingiberaceae family, known locally as "Waan chak mod luk" in Thailand. On the basis of pharmacological effect, the ethyl acetate extract has been shown to effectively reduce plasma triglyceride and cholesterol (6). This effect was associated with an increased biliary secretion of bile acid, a decreased secretion of cholesterol and phospholipids, and a lowered bile lithogenic index (6-7). However, those studies focused on the prevention of atherosclerosis. *C. comosa* supplementation was started simultaneously with the induction of hypercholesterolemia,

which does not resemble the clinical situation of patients whose hypercholesterolemia or atherosclerosis is already present. In this study, we investigated the effects of the crude powder of *C. comosa* on already established atherosclerotic lesions and endothelial dysfunction characterized by reduced endothelium-dependent relaxations in hypercholesterolemic rabbits.

Methods

Forty-four male New Zealand White rabbits weighing 1.5-2.00 kg were used in this study. The experiment on animals was followed the National Laboratory Animal Center, Thailand. After 2 weeks of adaptation, the rabbits were exposed to dietary treatment, commercial diet preparation of C.P. Company, Thailand for a period of 4 weeks and 16 weeks. The diet and water supplied was unrestricted throughout the experimental periods.

The rabbits were randomly divided into 6 groups. Rabbits in the first two groups were fed normal rabbit chow and plain tap water throughout the experimental period of 4 weeks and 16 weeks, respectively, and served as the control groups. The other 4 groups were fed a diet containing 1% cholesterol for the first 4 weeks. One of these groups (cholesterol-4-weeks group, n=8) was studied after 4 weeks. The other groups were followed for the next 12 weeks and fed 0.5% cholesterol containing diet (cholesterol-16-weeks group, n=8), 0.5% cholesterol containing diet with 5 mg/day of simvastatin (simvastatin group n=8), 0.5% cholesterol containing diet with 400 mg/kg/day of crude powder of *C. comosa* (comosa group, n=8). Blood sample was obtained from the central ear vein at the beginning of the experiment and every 4 weeks thereafter. Plasma was separated by centrifugation at 3,500 rpm for 15 minutes and kept at -20 ^{0}C until the analysis of cholesterol concentrations. At the end of the 4th week and 16th week of feeding periods, aorta was isolated and immediately placed in fresh ice-cold Krebs buffer for preparation for the measurement of vascular endothelium-dependent and –independent relaxations. The left common carotid artery was removed and dissected free of adherent fat and fascia and used for morphometric analysis. The result express as percent of plaque formation.

The data were analyzed by SPSS version 11.5 software program (SPSS Inc., USA), and were analyzed using one-way analyzing of variance (one-way ANOVA). The significant different between mean group is at the level of P value<0.05.

Results

The plasma lipid profiles in all groups of rabbits were shown in table 1. During the first 4 weeks of 1% cholesterol induction, the plasma total cholesterol, LDL-C, HDL-C and triglyceride concentration showed significant increased when compared to the baseline but no significant differences among variable groups. When the diet was switched to 0.5% cholesterol for further 12 weeks, the plasma total cholesterol, LDL-C, HDL-C and triglyceride concentration in cholesterol-16-weeks group increased continuously throughout the experimental period. The rabbits in the simvastatin or comosa group showed significantly decreased of total-C and LDL-C concentration at weeks 12 and 16 when compared to cholesterol-16-weeks groups. The rabbits in the simvastatin group showed significant increased of HDL-C only at the week 8 and decreased of plasma triglyceride concentrations at the last week of experiment. Treatments with *C. comosa* had no effects on either plasma HDL-C or triglyceride concentrations.

The percentages of vasorelaxation to ACh and SNP in all groups were shown in figure 1. After 4 weeks of cholesterol feeding, endothelium-dependent relaxations to ACh were significantly diminished as compared to the control-4-weeks group, and were severely impaired by further 12 weeks of 0.5% cholesterol feeding. Treatment with 5 mg/day simvastatin or 400 mg/kg/day *C. comosa* partly, but not completely restored endothelium-

dependent relaxation to ACh. The percentage of vasorelaxation in the simvastatin and comosa groups were significantly decreased compared to control-4 weeks and control-16-weeks groups, and significantly increased as compared to the cholesterol-16-weeks groups, but had no significant difference when compared to the cholesterol-4-weeks group. All of the experimental groups of rabbits showed no significant differences in endothelium-independent relaxation in response to SNP. The plaque formation in left internal carotid arteries in all group were shown in figure 2. After 4 weeks of cholesterol feeding, the rabbits showed about 31% of total plaque area in the left internal carotid arteries. When the cholesterol-feeding was further continued for 12 weeks, plaque formation progressive to about 45%. Treatment with simvastatin or *C. comosa* decreased the progression of plaque formation about 60% and 30%, respectively.

Table 1 Plasma cholesterol, HDL-C, LDL-C, Triglycerides levels of the rabbits on various diets and treatments.

Groups	Time period (weeks)				
	0	4	8	12	16
Cholesterol (mg/dl)					
Control-4-weeks	49.3±3.5	45.3±3.5			
Cholesterol-4-wks	46.7±5.2	2105.7±32.6*			
Control-16-wks	46.0±4.9	46.3±4.7	40.3±4.4	43.3±3.3	41.3±9.0
Cholesterol-16-wks	47.3 ± 3.0	2193.8±250.4*	1950.8±254.3*	2113.0±162.3*	2135.3±169.3*
Simvastatin	48.5±2.9	2056.5±248.6*	1590.7±299.4*	1079.8±126.7*†	1095.8±136.0*†
Comosa	49.5±3.8	2054.7±369.1*	1482.6±213.2*	1587.6±205.7*†‡	1555.7±164.07*†‡
LDL-C (mg/dl)					
Control-4-wks	14.3±1.8	15.3±2.2			
Cholesterol-4-wks	14.3±2.9	1676.7±241.3*			
Control-16-wks	14.0±4.9	13.0±4.0	12.3±6.6	14.3±5.6	14.0±5.7
Cholesterol-16-wks	14.5±3.5	1755.2±328.4*	1373.3±139.9*	1718.3±104.0*	1816.7±83.3*
Simvastatin	14.3±2.7	1871.8±232.1*	1139.3±149.1*	898.3±53.4*†	878.8±94.2*†
Comosa	15.0±1.4	1620.6±329.6*	1268.8±215.1*	1362.5±190.3*†	1267.4±144.4*†‡
HDL-C (mg/dl)					
Control-4-wks	45.3±8.4	49.0±10.0			
Cholesterol-4-wks	44.7±2.4	393.3±52.1*			
Control-16-wks	42.0±12.5	41.7±7.5	43.3±9.0	45.7±3.5	40.0±11.4
Cholesterol-16-wks	41.5±6.0	425.0±78.0*	302.5±30.3*	429.8±13.9*	386.8±17.8*
Simvastatin	43.0±5.0	553.0±115.0*	491.0±79.5*†	343.0±16.8*†	384.0±83.7*
Comosa	41.9±2.8	557.9±91.6*	370.9±56.0*	391.0±13.8*‡	326.6±11.8*
Triglyceride(mg/dl)					
Control-4-wks	89.3±18.8	76.0±25.9			
Cholesterol-4-wks	87.3±37.4	137.0±22.6*			
Control-16-wks	74.3±5.2	72.0±7.0	73.7±17.7	77.3±17.0	73.0±7.0
Cholesterol-16-wks	76.3±16.2	132.3±22.3*	137.5±20.1*	129.5±23.0*	139.3±19.3*
Simvastatin	57.8±8.1	134.5±23.7*	102.8±42.6*	104.3±27.5*	88.0±16.4*†
Comosa	69.3±8.2	122.1±21.4*	97.1±23.9*	132.0±34.3*	113.7±18.7*

All values are means ± SEM, * P<0.05 compared to control group, †P<0.05 compared to cholesterol group, ‡ P<0.05 compared to simvastatin group

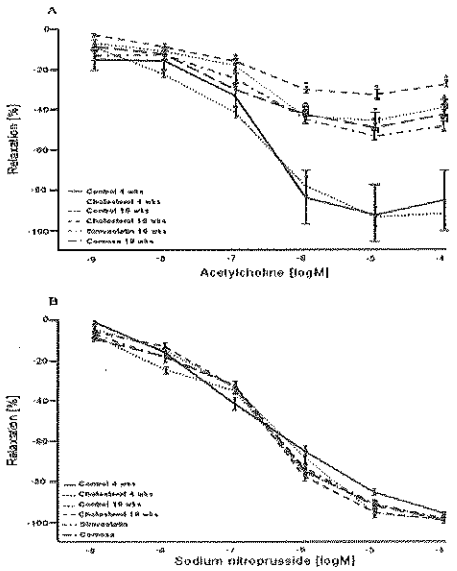

Figure 1 Concentration response curves of vasorelaxation to acethylcholine (A) and sodium nitroprusside (B) in isolated aortas from rabbits in control and cholesterol-fed diet for 4 or 16 weeks with or without supplementation with simvastatin or crude powder of *C.comosa.*
Data are means ± SEM
* P<0.05 compared to control group
† P<0.05 compared to cholesterol group

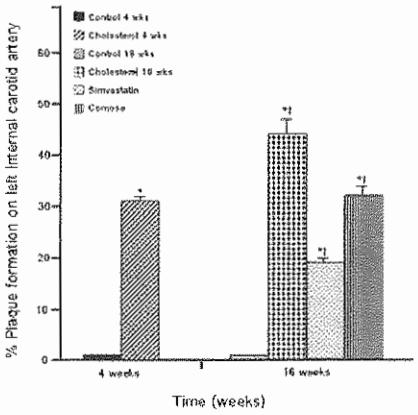

Figure 2 Total plaque areas in the left internal carotid arteries of the rabbits on various diets and treatments. Data are means ± SEM * P<0.05 compared to control group, † P<0.05 compared to cholesterol group

Disscussion

The results in this study demonstrate that the crude powder of *C. comosa* Roxb. decresed plasma total cholesterol, LDL-C, restored endothelial function and slowed the progression of intimal plaque formation in carotid arteries of treated rabbits. The plasma lipid-lowering effects of *C. Comosa* may result from choloretic activity (6). One of the most active chemical compound in *C. comosa* is a glucoside of phloracetophenone or 2,4,6 trihydroxyacetophenone (THA) (7). THA lowers plasma cholesterol by an increasing hepatic cholesterol 7α-hydroxylase activity, a key enzyme in the conversion of cholesterol to bile acid and excreted via biliary secretion (7). In addition, it is able to stimulate bile secretion with a high bile flow rate and high bile salt output (7). The hexane extract of *C. Comosa* has demonstrated to possess estrogenic activity. It has been reported that estradiol could increase nitric oxide bioactivity acutely through a stimulation of endothelial nitric oxide synthase activity and chronically through a decreased breakdown of nitric oxide, as a consequence of a decreased production of reactive oxygen species (8).

Conclusion

Our study shows that *C. comosa* induces anti-atherosclerotic effects in cholesterol-fed rabbits at degrees comparable to those of simvastatin. It restored endothelium-dependent vasodilator function and reduced the progression of atherosclerotic lesions even though treatment was initiated 4 weeks after the induction of hypercholesterolemia which the rabbits showed the pre-established endothelial dysfunction and plaque formation. This finding may provide a therapeutic strategy for atherosclerosis which requires further experimental and clinical studies.

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Original article **P29**

Inhibition of Neutrophil Functional Responsiveness by Polyphenolic Compounds From *Polyalthia glauca* Extracts

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Abstract

Two new purified lignan compounds (compound 133 and 137) isolated from methanolic extract of *Polyalthia glauca* exhibited anti-inflammatory activities in the EPP-induced mouse ear edema model (unpublished data). The purposes of this study were to investigate the effects of the two purified compounds from *Polyalthia glauca* extract on adhesion molecule expression and human neutrophil responsiveness. The effects of compound 133 and 137 on fMLP-induced L-selectin shedding and an increase in MAC-1 expression on the neutrophils were assessed using flow cytometry. Human neutrophil functional responsiveness was determined by spectrophotometrically measuring fMLP-induced chemotaxis, superoxide anion generation (SAG), MPO release. Neutrophil viability was assessed by trypan blue exclusion and XTT cytotoxicity assay. The free-radical scavenging properties of the two compounds were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Compound 133 (10-100 μM) significantly inhibited L-selectin shedding, while compound 137 did not. Both 133 and 137 had no effect on fMLP-induced increased in MAC-1 expression. Compound 133 and 137 exhibited intermediate radical-scavenging activity when compared to trolox. Compound 133 and 137 concentration-dependently inhibited fMLP-induced SAG, chemotaxis, and MPO production in neutrophils. These finding suggest that the two purified compounds from *Polyalthia glauca* extract exert a specific action on adhesion receptor expression in neutrophils and inhibit some neutrophil functional response, which might account, at least in part, for their anti-inflammatory activities.

Keywords: *Polyalthia glauca*, Neutrophil functional responsiveness, L-selectin

Introduction

Acute inflammation is characterized by an accumulation of polymorphonuclear cells (PMNs), generation of reactive oxygen species and granule enzymes (1), subsequent apoptosis of PMNs, and finally phagocytosis of apoptotic cells by macrophages. The compounds isolated from *Polyalthia glauca,* which is commonly known as "Tara" in the south of Thailand, are alkaloids, terpenoids and lignans. The crude methanolic extract and the two purified lignan compounds, compound 133 and 137, from *Polyalthia glauca* extract exhibited anti-inflammatory activities which were found to be more potent than that of phenylbutazone in the mouse ear edema model (unpublished data). In this study, the effects of compound 133 and 137 on neutrophil functional responsiveness were investigated in order to elucidate the cellular mechanism of their anti-inflammatory activities.

Materials and Methods

DPPH scavenging activity: The free radical scavenging activities of compound 133 and 137 were determined with 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) in methanol(2). Absorbance at

515 nm was measured spectrophotometrically for 10 min and the scavenging activity was calculated as a percentage of radical reduction. Trolox (6.25-200μM) was used as a reference compound.

Preparation of human neutrophils: Human neutrophils were isolated by Percoll density gradient centrifugation. Venous blood obtained from healthy donors, was mixed with an equal volume of Percoll, and the mixture centrifuged at 280g for 20 min. After centrifugation, PMN were harvested and washed. Any contaminating red cells were removed. The cells were >99% viable as determined by trypan blue exclusion and were resuspended as required.

Cytotoxicity assay: Human neutrophils were incubated with various concentrations of compound 133 and 137 for 4 h, then XTT was added and incubated at 37 ◦C for an additional 4 h. The XTT formazan product was measured spectrophotometrically at 450 nm(3).

Determination of Neutrophil Chemotaxis: The method for assessment of human neutrophil chemotaxis is the measurement of neutrophil migration across the polyvinylcarbonate filter using chemotaxis chamber(4). The bottom wells of the chamber were filled with fMLP. The top plate with the installed filter was placed onto the filled bottom plate, and upper wells were filled with neutrophils treated with the various concentrations of compound 133 and 137. After incubation, the filter removed, washed, fixed and stained with DiffQuikTM. Chemotaxis was quantified by measuring absorbance at 550 nm. Indomethacin was used as a reference compound.

Determination of Superoxide Anion Generation (SAG): Neutrophil SAG is determined by spectrophotometric evaluation of the reduction of ferricytochrome C to ferrocytochrome C in the presence of cytochalasin B(5). Neutrophils resuspended in PBS containing cytochrome C/cytochalasin B were preincubated with various concentrations of compound 133 and 137 before stimulating with fMLP. The reaction was terminated by immersing the tubed in an ice bath followed by centrifugation. The absorbance of the supernatant from each tube were measured at 550 nm. Indomethacin was used as a standard reference compound.

Determination of Myeloperoxidase (MPO) production: Neutrophils were preincubated with compound 133 and 137 at various concentrations before stimulating with fMLP, then incubated for 10 min. After centrifugation, supernatants were incubated with the reaction mixture of 3,3',5,5'-Tetramethylbenzidine (TMB) supplemented with H_2O_2. The reaction was then stopped by adding H_2SO_4 and absorbance was measured spectrophotometrically at 450 nm(6). Indomethacin was used as a reference compound.

Determination of adhesion molecule expression on human neutrophils: Neutrophils were preincubated with compound 133 and 137 at various concentrations in incubator before stimulating with fMLP. After incubation, neutrophils were stained with PE Mouse Anti-Human CD62L or APC Mouse Anti-Human CD11b/Mac-1 on ice. The analysis of immunofluorescence as a measure of Mac-1 (CD11b/CD18) and L-selectin (CD62L) surface expression was perform on a FACS Calibur flow cytometer (7).

Results

DPPH scavenging activity

Both purified compounds exhibited intermediate radical scavenging activities (EC_{50} of 77.9±15.3μM and of 79.9±3.8μM for compound 133 and 137, respectively) compared with Trolox (EC_{50} of 52.7±6.23 μM) which showed high radical scavenging activity.

Cytotoxic effects

Neutrophil viability was not affected by the two purified compounds up to the 10^{-4} M (IC_{50} > 500 μM for compound 133 and IC_{50} > 1000 μM for compound 137, n=6).

Neutrophil Superoxide Anion Generation (SAG)

Compound 133 exerted stronger inhibitory effects on fMLP-induced human neutrophil SAG than that of compound 137 (IC_{50} of 34.2±4.6 μM, and of 61.4±14.6 μM, for compound 133 and 137, respectively) (Figure1). Indomethacin (0.1-100μM) caused strong inhibition of fMLP-induced SAG in human neutrophil, with IC_{50} of 2.5± 0.2, n=5.

Neutrophil chemotaxis

Compound 133 and 137 significantly suppressed fMLP-induced human neutrophil chemotaxis in a concentration-dependent manner with an IC_{50} of 34.6±1.8 μM and an IC_{50} of 42.1±2.8 μM, respectively (Figure2). Indomethacin (0.1-100μM) caused strong inhibition of fMLP-induced human neutrophil chemotaxis, with IC_{50} of 2.3.± 0.3, n=5.

Neutrophil MPO production

The two pure compounds 133 and 137 caused significantly inhibition of fMLP-induced MPO production in human neutrophils with an IC_{50} of 43.6±1.3 μM and an IC_{50} of 71.6±13.8 μM, respectively. The pure compound 133 exert a stronger inhibition than that of the pure compound 137. Indomethacin (0.1-100μM) caused strong inhibition of fMLP-induced MPO production in human neutrophil, with IC_{50} of 2.3± 0.2, n=5.

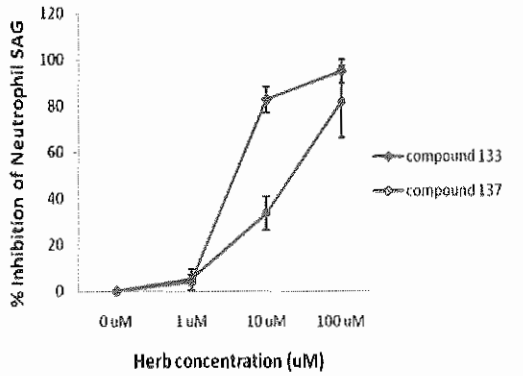

Figure 1. Inhibitory effects of purified compound 133 and137 on fMLP-induced SAG in human neutrophils. SAG was measured measured by the reduction of ferricytochrome C at A_{550} nm . Results are mean±S.E.M of 5 experiments using cells from different donors.

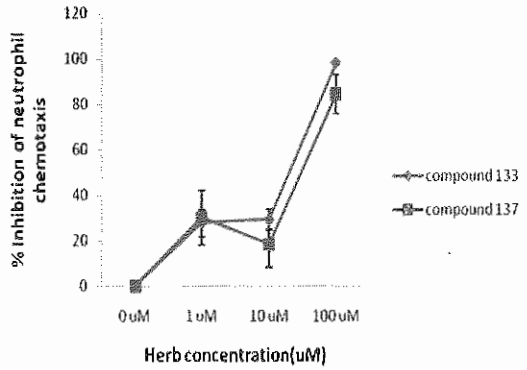

Figure 2 Inhibitory effects of purified compound 133 and 137 on fMLP-induced chemotaxis in human neutrophils. Chemotaxis was quantified spectrophotometrically at 550 nm. Results are mean± S.E.M of 5 experiments using cells from from different donors.

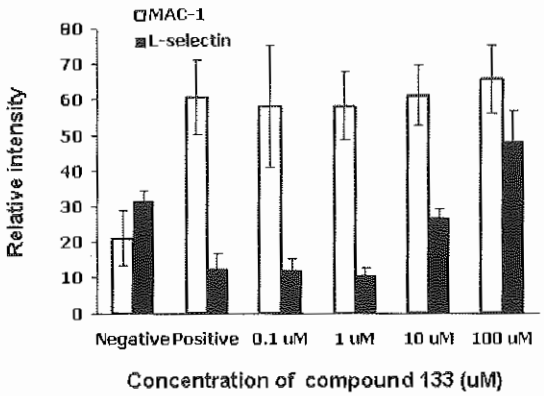

Figure 3 Effects of the pure compound 133 on the fMLP-stimulated surface expression of MAC-1 and L-selectins on human neutrophils. Positive ; fMLP- stimulated adhesion molecule expression without pure compound. Results are the mean±S.E.M of 4 experiments with cell preparation from different donors.

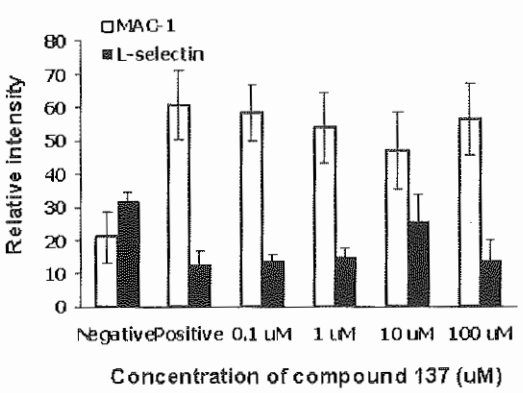

Figure 4 Effects of the pure compound 137 on the fMLP-stimulated surface expression of MAC-1 and L-selectin on human neutrophils. Positive; fMLP-stimulated adhesion molecule expression without pure compound. Results are the mean± S.E.M of 4 experiments with cell preparation from different donors.

Neutrophil adhesion molecules expression

Activation of neutrophil by fMLP caused L-selectin shedding and increase in MAC-1 expression. Compound 133 at the concentration of 10 μM and100 μM significantly inhibited

shedding of L-selectin but had no effect on Mac-1 expression. Compound 137 did not affect both fMLP-induced L-selectin shedding and an increase in Mac-1 expression

Discussion and Conclusion

Neutrophil recruitment into inflamed tissue proceeds via multi-step processes. The first contact of neutrophils with endothelium is mediated by selectins and their counterreceptors, followed by rolling of neutrophils along the endothelial wall of postcapillary venules and β_2-integrin (Mac-1) -mediated arrest. During neutrophil activation, L-selectin, which initially has a high basal expression, is rapidly down-regulated (shed) and causes up-regulated Mac-1 (CD11b/Cd18) surface expression. In addition to activation of neutrophils by ligation with chemoattractants, integrins and selectin ligands are also able to signal into the cell, where they initiate neutrophil extravasation, promote cytoskeletal rearrangement, and ultimately induce superoxide production and degranulation (8). Compound 133 (10-100 μM) significantly inhibited shedding of L-selectin, while compound 137 did not. Both 133 and 137 had no effect on fMLP-induced increased in MAC-1 expression. Activation of neutrophils by fMLP caused neutrophil chemotaxis, release of O_2^- and degranulation of neutrophil granules. Pre-treatment of neutrophils with compound 133 and 137 significantly inhibited fMLP-induced chemotaxis, SAG and MPO production. Compound 133 showed stronger inhibitory effects on fMLP-induced all neutrophil functional responsiveness mentioned above than those of compound 137. Compound 133 and 137 possess intermediate radical scavenging activities, which may be attributed to their inhibitory effects on fMLP-induced SAG in neutrophil. Taken together, these finding suggest that the two purified compounds from *Polyalthia glauca* extract exert a specific action on adhesion receptor expression in neutrophils and inhibit some neutrophil functional response, which might account, at least in part, for their anti-inflammatory activities.

Acknowledgement

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Original article **P30**

The Effect of Dietary Supplement derived from *Scaphium scaphigerum* (G.Don) Fruit on Chromosomal Damage in Rat Bone Marrow Cells

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Abstract

Scaphium scaphigerum fruit (in Thai, "Phungthalai" or "Samrong") has been used as a traditional medicine in many Asian countries. Therefore, it is interested to evaluate the toxic effect of this fruit, particularly its genotoxic effect. In this study, the effect of dietary supplement derived from *Scaphium scaphigerum* fruit (SCD) on the chromosome of rat bone marrow cells was investigated. Both sexes of Wistar rats were orally administered the SCD at a dose of 5,000 mg/kg BW. Twenty-four hours after treatment, the bone marrow cells were isolated from their femora for chromosomal analysis. The results of this study demonstrated that the SCD at a given dose did not cause chromosomal aberrations, compared to the control.

Keywords: *Scaphium scaphigerum*; Genotoxic effect; Chromosomal analysis

Introduction

Recently, dietary supplement consumption is a new trend for the people who care about their health. Dietary supplement has been widely believed to enhance the immune system in human body. Phungthalai or Samrong is the fruit of *Scaphium scaphigerum* (G.Don) which belongs to the plant family *Sterculiaceae*. This plant is distributed throughout tropical and sub-tropical regions of the world. It is mostly found in South East Asian countries, especially in Thailand, Cambodia, Malaysia and Indonesia. It is a plant growing to heights of 4-5 meter and the characteristic of its fruit is a small brownish oval shape. The fruit of this plant has been used in folklore medicine to treat many diseases such as fever, diarrhea, kidney and large intestine disorders.

Although, this fruit has been used for a long time but some toxicity reports or published data on its toxicity and safety has been limited. The aim of this study was to investigate the effect of *Scaphium scaphigerum* fruit on chromosomal aberrations by using cytogenetic method.

Materials and methods

Animals

Both sexes of Wistar rats, 5–7 weeks old and weighting 140–160 g were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. Eighteen rats were divided into the negative control, treatment and positive control groups. Each group contained three male and three female rats. They were acclimatized to the laboratory environment for one week prior to experimentation. The rats were randomly chosen using the simple random sampling method and identified numbers by tail labeling. They were fasted for 16 hrs prior to the dosing with the test material while drinking water was available *ad libitum*.

Chemicals and selection of dose

Based on the Test Guideline No. 475 "Genetic Toxicology : *In vivo* Mammalian Bone Marrow Cytogenetic Test – Chromosomal Analysis" of OECD Guidelines for Testing of Chemicals 1997 (3). The SCD was freshly prepared in the form of suspension in distilled water and mixed well before oral administration for 14 consecutive days. The rats of the negative control group were dosed with distilled water at the equivolume as the treated group.

Cyclophosphamide (CP) was freshly prepared by dissolving in sterile distilled water at a dose of 50 mg/kg body weight. CP was injected intraperitoneally as a single agent to the rats as the positive control. The animals were sacrificed under CO_2 anesthesia 24 h after mutagen administration.

Bone marrow cells preparation

Ninety minutes before sacrifice, all animals were injected intraperitoneally with colchicine at the dose of 3.5 mg/kg body weight. Their femurs were removed and the femoral marrow cells were flushed out with HBSS medium for chromosomal analysis (4). Bone marrow cells preparations were made by hypotonic solution and fixative. All slides were coded and stained with 10% Giemsa (1).

Scoring criteria

In this study, metaphase cells with one or more chromosome aberrations were scored from 50 well-spread metaphases per animal at random. The types of aberration were scored and recorded strictly in accordance with the method of Ito & Ito. The severity of chromosome aberrations included the aberrations with break, exchange and multiple aberrations, but not the aberrations with gap (2).

Statistical analysis

This study was analyzed and expressed as mean ± SEM. Each treatment group was compared to the control group. The Student's t-Test was done to observe the significant differences between individual treated group and the control groups. The level of significance was established at $p < 0.05$.

Results

The results of chromosomal analysis in rat bone marrow cells at metaphase stage after receiving the SCD at a dose of 5,000 mg/kg BW by oral administration for 14 consecutive days were summarized in Table 1.

Table 1. Chromosomal analysis of rat bone marrow cells receiving the SCD for 14 consecutive days.

Group	Treatment	[a,b]M.I (%)	[a,c]Type of chromosomal aberration			Total cells with aberration
			Break	Exchange	Multiple aberrations	
1	Distilled water	6.03±0.31	0	0	0	0
2	SCD	5.54±0.20	0	0	0	0
3	CP	2.86±0.09*	2.17 ± 0.72*	2.67 ± 1.04*	8.50 ± 1.59*	13.33 ± 1.66*

[a] Data were expressed as Mean ± SEM, n= 6 and analyzed statistically by Student's t-Test.
[b] Mitotic Index (M.I.) was based on 2,000 cells per animal.
[c] Chromosome aberration was based on 50 cells per animal.
* p<0-05; Significant difference from Group 1 and 2.

The percentage of Mitotic Index (%M.I.) and type of chromosome aberrations of rat bone marrow cells treated with the SCD were shown in Fig. 1 and Fig.2, respectively. The results demonstrated that %M.I. of the SCD treated group did not show significant difference from negative control group. There was no significant difference in chromosomal aberration between treated group and negative control group. This means that the SCD at a given dose did not show cytotoxic effect on rat bone marrow cells and did not cause chromosomal aberrations.

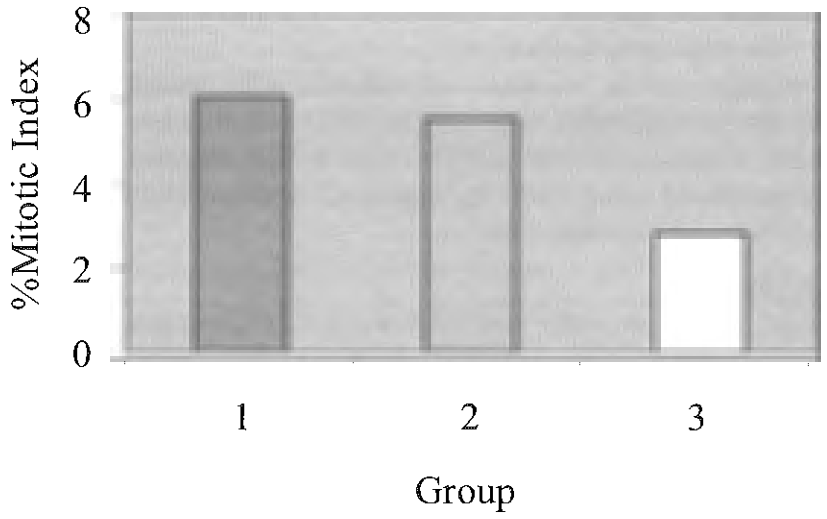

Figure 1. The %M.I. of rat bone marrow cells treated with the SCD for 14 consecutive days.

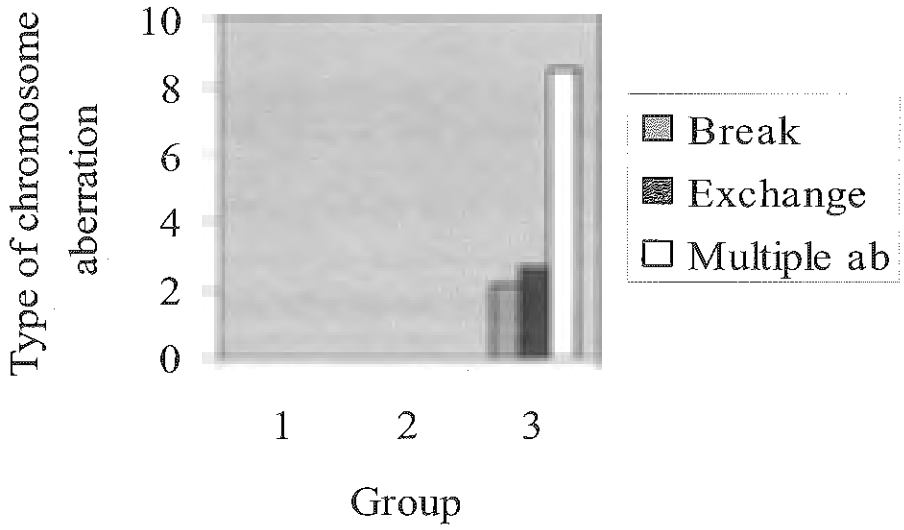

Figure 2. The type of chromosomal aberrations of rat bone marrow cells treated with the SCD for 14 consecutive days.

Conclusion

In this study, we concluded that dietary supplement derived from *Scaphium scaphigerum* (G.Don) fruit at a dose of 5,000 mg/kg BW might not show cytotoxic effect on rat bone marrow cells and might not cause any chromosomal aberrations.

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