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สารจากนายกสมาคมเภสัชวิทยาแห่งประเทศไทย

เรียน สมาชิกสมาคมเภสัชวิทยาและผู้เข้าร่วมประชุมทุกท่าน

ขอต้อนรับทุกท่านเข้าร่วมประชุมวิชาการประจำปีครั้งที่ 30 ของสมาคมเภสัชวิทยาแห่งประเทศไทย การประชุมครั้งนี้ ได้รับความเอื้อเฟื้อจากสมาชิกภาควิชาเภสัชวิทยา ของคณะแพทยศาสตร์ คณะเภสัชศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ร่วมเป็นเจ้าภาพจัดการประชุมครั้งนี้ โดยมี รศ.พญ.สุมนา ชมพูทวีป เป็นประธาน ภายใต้หัวข้อการประชุมว่า From Pharmacology to National Policy (จากความรู้ทางเภสัชวิทยาสู่นโยบายยาแห่งชาติ) การประชุมครั้งนี้ คณะผู้จัดการประชุมฯ ได้เรียนเชิญ ศ.นพ.ธาดา ยิบอินซอย ประธานคณะกรรมการการพัฒนาระบบประกันสุขภาพแห่งชาติ มาเป็นองค์ปาฐกถา รศ.จิรวัฒก์ สดาวงศ์วิวัฒน์ ครั้งที่ 15 และผู้ทรงคุณวุฒิมาให้ความรู้ทบทวนความเป็นปัจจุบันของกลุ่มยารักษาโรคซึมเศร้า กลุ่มยารักษาโรคหัวใจและหลอดเลือด การประเมินคุณภาพและประสิทธิภาพของยา ที่เป็นชีววัตถุตลอดจนการอภิปรายกลุ่ม เรื่อง ข้อพึงระวังใน การนำ stem cell มาใช้ในทางการแพทย์ นอกจากนี้ยังมีการนำเสนอผลงานวิจัยของนักเภสัชวิทยารุ่นใหม่ นักศึกษาบัณฑิต และการมอบรางวัลผลงานวิจัยดีเด่นประจำปี ในนามของสมาคมฯ กระผม ใคร่ขอขอบคุณวิทยากร คณะกรรมการจัดการประชุม สมาชิก นักศึกษา ผู้ให้การสนับสนุนทั้งจากหน่วยงานภาครัฐและเอกชน และผู้เข้าร่วมประชุมทุกท่านที่ได้มีส่วนทำให้การประชุมครั้งนี้ สำเร็จบรรลุจุดประสงค์ เป็นที่พอใจทุกประการ

ดร. อุดม จันทรารักษ์ศรี

นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

สารจากประธานจัดงานประชุมวิชาการประจำปีครั้งที่ 30

เรียน ท่านสมาชิกชาวเภสัชวิทยาและผู้เข้าร่วมประชุมทุกท่าน

การประชุมวิชาการประจำปีของสมาคมเภสัชวิทยาแห่งประเทศไทย เป็นกิจกรรมหลักของสมาคมฯ เพื่อเป็นเวทีสำหรับติดตามความก้าวหน้าของวิชาการสาขาเภสัชวิทยา แลกเปลี่ยนความรู้ ความคิดเห็นและประสบการณ์ระหว่างนักเภสัชวิทยาและนักวิชาการสาขาอื่นที่เกี่ยวข้อง นอกจากนี้ยังเป็นโอกาสอันดีที่สมาชิกและผู้สนใจจะได้พบปะสังสรรค์ เชื่อมความสามัคคี อันจะนำไปสู่ความร่วมมือด้านการเรียนการสอนและการวิจัยในอนาคต เพื่อให้สถาบันที่จัดการเรียนการสอนและการวิจัยทางเภสัชวิทยามีความเข็มแข็งและก้าวไปด้วยกันฉันท์เพื่อน-พี่-น้อง และทำให้วิชาการสาขาเภสัชวิทยาในประเทศไทยมีความก้าวหน้าและมีความทันสมัยเป็นที่ยอมรับในระดับสากล

ดิฉันใคร่ขอแสดงความขอบคุณท่านผู้เข้าร่วมประชุมทุกท่าน วิทยากร และผู้ให้การสนับสนุนทุกฝ่าย หากมีข้อผิดพลาดและข้อบกพร่องประการใด ที่อาจเกิดขึ้น ดิฉันต้องขออภัยไว้ล่วงหน้า และขอรับคำแนะนำด้วยความยินดี

รศ.พญ.สุมนา ชมพูทวีป
ประธานกรรมการจัดประชุมฯ

คำปราศรัยของ ศ.ดร.คุณหญิงสุชาดา กีระนันทน์ อธิการบดี จุฬาลงกรณ์มหาวิทยาลัย เนื่องในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 30 สมาคมเภสัชวิทยาแห่งประเทศไทย ในวันพฤหัสบดีที่ 27 มีนาคม 2551

ท่านประธานคณะกรรมการจัดประชุมฯ ท่านนายกสมาคมเภสัชวิทยาแห่งประเทศไทย และท่านผู้มีเกียรติทุกท่าน

ดิฉันรู้สึกเป็นเกียรติและยินดีอย่างยิ่งที่ได้มีโอกาสมาเป็นประธานในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 30 ของสมาคมเภสัชวิทยาแห่งประเทศไทยในวันนี้ ขอแสดงความยินดีที่สมาคมเภสัชวิทยาฯ ได้ก่อตัวและประสบความสำเร็จในการดำเนินงานมาโดยตลอด

ปัจจุบัน วิชาการสาขาต่างๆ และเทคโนโลยีได้ก้าวหน้าไปอย่างรวดเร็ว โดยเฉพาะอย่างยิ่งการวิจัยและพัฒนายาใหม่ๆ ออกสู่ท้องตลาดเป็นจำนวนมาก และโดยที่ยุคนี้จัดว่าเป็นยุคโลกไร้-พรมแดน การประชุมวิชาการเป็นวิธีการหนึ่งที่ทำให้นักวิชาการสามารถติดตามรับทราบข้อมูลวิชาการและเทคโนโลยีที่ก้าวรุดหน้าได้รวดเร็วขึ้น ซึ่งจะทำให้นักเภสัชวิทยาและบุคลากรด้านการแพทย์ได้พัฒนาตนเอง เป็นประโยชน์ต่อทั้งหน่วยงานและประเทศชาติ

บัดนี้ ได้เวลาอันสมควรแล้ว ดิฉันขอเปิดการประชุมวิชาการครั้งที่ 30 ของสมาคมเภสัชวิทยาแห่งประเทศไทย และขออวยพรให้การประชุมครั้งนี้บรรลุตามวัตถุประสงค์ และสำเร็จสมดังเจตนารมณ์ที่ตั้งไว้ทุกประการ

ศ.ดร.คุณหญิงสุชาดา กีระนันทน์
อธิการบดี จุฬาลงกรณ์มหาวิทยาลัย

บรรณาธิการแถลง

เรียนท่านผู้เข้าร่วมประชุมและสมาชิกสมาคมเภสัชวิทยาแห่งประเทศไทย

วารสารฉบับรวบรวมบทความวิจัยของการประชุมวิชาการประจำปี 2551 ของสมาคมเภสัชวิทยาแห่งประเทศไทยฉบับนี้ ยังคงนำเสนอผลงานวิจัยที่ให้รายละเอียดมากขึ้นนอกจากบทคัดย่อ แต่ไม่ยาวจนเกินไป เพื่อตอบสนองความต้องการของนิสิตนักศึกษาบัณฑิตศึกษาในหลักสูตรเภสัชวิทยาตลอดจนสาขาอื่นที่เกี่ยวข้อง สำหรับปีนี้ มีผลงานในรูปแบบโปสเตอร์เป็นจำนวนมากกว่าทุกปีที่ผ่านมา

การประชุมในปีนี้แน่นไปด้วยเนื้อหาที่น่าสนใจ ไม่ว่าจะเป็นนโยบายระดับชาติของการใช้ยาที่จัดขึ้นในวันแรก ในวันที่สองจะเกี่ยวข้องกับยาที่เป็น biological products เริ่มด้วย "biosimilars" ที่กำลังได้รับความสนใจเป็นอย่างมากเพราะว่าเกี่ยวข้องกับคุณภาพของยาในกลุ่มนี้ ซึ่งย่อมมีผลต่อประสิทธิภาพของยาในการรักษา ตามมาด้วยประสบการณ์ทางคลินิกของยาเหล่านี้ สำหรับ luncheon symposium ที่ทางบริษัทเซอร์เวีย (ประเทศไทย) จัดมานำเสนอทั้งสองวัน ก็ยังคงเป็นหัวข้อที่น่าสนใจ ไม่ว่าจะเป็นการศึกษาการใช้ยาในระบบหัวใจและหลอดเลือดแล้ว ยังมีเนื้อหาใหม่ๆที่เกี่ยวกับโรคซึมเศร้า โรคของคนรุ่นใหม่ที่กำลังมาแรงจนมียาออกมาใหม่มากมายจนน่าแปลกใจในช่วงนี้

น่าเสียดายที่เรามีปัญหาในเรื่องของเวลาที่มีจำกัดจนไม่สามารถลงเนื้อหาได้หมด เนื่องจากท่านวิทยากรหลายท่านไม่สามารถส่งมาให้ทันเวลาในการจัดทำ proceeding อย่างไรก็ตาม ท่านสามารถติดตามเนื้อหานี้ได้จากเว็ปไซต์ของสมาคม ซึ่งคงใช้เวลาไม่นานนักหลังสิ้นสุดการประชุม สำหรับท่านสมาชิกสมาคมฯ ที่พลาดจากการประชุมวิชาการในครั้งนี้ยังคงได้รับวารสารฉบับพิเศษฉบับนี้ได้ตามปกติ

ขอขอบพระคุณทุกท่านที่มีส่วนช่วยเหลือการจัดทำวารสารและการจัดประชุมวิชาการในครั้งนี้ ขอบคุณเพื่อนๆ พี่น้องในภาคธุรกิจเอกชนที่สนับสนุนกิจกรรมของสมาคมฯมาตลอด และขอขอบพระคุณทุกท่านที่ให้ความร่วมมือส่งบทความมาให้ทันเวลา ทำให้วารสารฉบับนี้หนากว่าทุกปี

รศ. ดร. สุพัตรา ศรีไชยรัตน์

บรรณาธิการ

สรุปผลงานของคณะกรรมการบริหารสมาคมเภสัชวิทยาแห่งประเทศไทย

วาระ 2549-2551

เมษายน 2550 - มีนาคม 2551

1. จัดประชุมวิชาการร่วมกับสถาบันการศึกษาต่อเนื่องทางเภสัชศาสตร์ สำนักงานคณะกรรมการอาหารและยา เพื่อการบริการวิชาการแด่บุคลากรทางด้านสาธารณสุข ณ สำนักงานคณะกรรมการอาหารและยา ในเรื่อง

1.1 "Melatonin and Depression : New concept in therapeutic and management" โดย ศ.นพ.อนันต์ ศรีเกียรติขจร ในวันพฤหัสบดีที่ 17 พฤษภาคม 2550 เวลา 10.00 – 12.00 น. ณ ห้องประชุมชั้น 6 อาคาร 4 สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข จังหวัดนนทบุรี

1.2 "การพัฒนาระบบยาในการบริหารโรคเรื้อรัง" โดย นพ.ชูชัย ศรชำนิ ในวันอังคารที่ 10 กรกฎาคม 2550 เวลา 10.00 – 12.00 น. ณ ห้องประชุมชั้น 6 อาคาร 4 สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข จังหวัดนนทบุรี

1.3 "เซลล์ต้นกำเนิด" (ความคาดหวังในการใช้เซลล์ต้นกำเนิดในการรักษาโรค) โดย ผศ.นพ.ดร.นิพัญจน์ อิศรเสนา ณ อยุธยา ในพฤหัสบดีที่ 11 ตุลาคม 2550 เวลา 10.00 – 12.00 น. ณ ห้องประชุมชั้น 6 อาคาร 4 สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข จังหวัดนนทบุรี

1.4 "ทิศทางการรักษาโรคกระดูกพรุน" โดย ศ.นพ.นิมิต เตชไกรชนะ วันพฤหัสบดีที่ 6 มีนาคม 2551 เวลา 10.00-12.00 น. ณ ห้องประชุมชั้น 6 อาคาร 4 สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข จังหวัดนนทบุรี

1.5 "มาตรการประกันคุณภาพและประเมินประสิทธิภาพของสารชีววัตถุ (Measure Toward Quality Assurance of Biologicals)" โดย อ.พญ.อรณี ตั้งเผ่า ในวันอังคารที่ 25 มีนาคม พ.ศ. 2551เวลา 10.00 – 12.00 น. ณ ห้องประชุมหลวงวิเชียรแพทยสมาคม อาคาร A ชั้น 2 สำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุข จังหวัดนนทบุรี

2. จัดงานแสดงมุทิตาจิตแด่อาจารย์เกษียณอายุราชการ ในวันศุกร์ที่ 12 ตุลาคม 2550 เวลา 11.00 – 13.0 น. ณ ห้อง VIP 2 สโมสรทหารบก – วิภาวดี

3. จัดประชุมกลุ่มย่อยเรื่อง"การเรียนการสอนและการออกข้อสอบวิชาเภสัชวิทยา "ยากลุ่มที่ออกฤทธิ์ต่อระบบประสาทส่วนกลางและจิตประสาท" ในวันศุกร์ที่ 2 พฤศจิกายน 2550 เวลา 13.00-16.00 น. ณ ห้อง PR-501 คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล โดยที่ประชุมได้จัดทำเป็นหัวข้อของเนื้อหาที่ควรจัดให้มีการเรียนการสอนสำหรับยาในระบบนี้ให้สอดคล้องกับความต้องการของแพทยสภา และนำขึ้นเว็บไซต์สมาคมฯ เพื่อแผยแพร่ให้ทราบโดยทั่วกัน พร้อมเปิดกระทู้ในเว็บบอร์ด เพื่อการเสนอแนะ

4. จัดประชุมระดมความคิดเพื่อหาแนวทางการสร้างเครือข่ายงานวิจัยทางด้าน pharmacogenomic ในวันศุกร์ที่ 29 กุมภาพันธ์ 2551 เวลา 11.30 น. ณ ห้อง PR-501 ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

5. จัดทำจุลสารสมาคมเภสัชวิทยาแห่งประเทศไทยผ่านเว็บไซต์สมาคมฯ www.phartherst.org

- ฉบับที่ 2/2550 ประจำเดือน กุมภาพันธ์ 2550
- ฉบับที่ 3/2550 ประจำเดือน มีนาคม 2550

6. จัดทำวารสารสมาคมเภสัชวิทยาแห่งประเทศไทยอย่างต่อเนื่อง

7. จัดทำโครงการเผยแพร่ข้อมูลเกี่ยวกับกิจกรรมบัณฑิตศึกษาผ่านทางเว็บไซต์สมาคมเภสัชวิทยาแห่งประเทศไทย www.phartherst.org

8. สร้างกลุ่มเครือข่ายวิชาการสมาคมเภสัชวิทยาแห่งประเทศไทย เพื่อการพัฒนางานด้านวิชาการและเผยแพร่ข้อมูลข่าวสารด้านเภสัชวิทยา แก่คณาจารย์ นิสิตบัณฑิตศึกษา สมาชิก และผู้สนใจ

9. จัดทำคลังข้อมูล email addressของสมาชิก นิสิตบัณฑิตศึกษาและกลุ่มเครือข่ายวิชาการสมาคมฯ

10.จัดประชุมวิชาการประจำปีครั้งที่ 30 ร่วมกับ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ คณะเภสัชศาสตร์ คณะสัตวแพทยศาสตร์ คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย หัวข้อเรื่อง From Pharmacology to National Drug Policy ระหว่างวันที่ 27-28 มีนาคม 2551 ณ ห้องประชุมสี สิริสิงห อาคารสมเด็จย่า ชั้น 2 คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

รายนามวิทยากร

ศ.นพ.ธาดา ยิบอินซอย	คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานรินทร์
ศ.พญ.สยมพร ศิรินาวิน	ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี
ภญ.อรวรรณ เกตุเจริญ	ฝ่ายเภสัชกรรม โรงพยาบาลจุฬาลงกรณ์
ผศ.นพ.พิสนธิ์ จงตระกูล	ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
อ.นพ.ยงเกษม วรเศรษฐการกิจ	ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
รศ.นพ.ดร.ศุภนิมิต ทีฆชุณหเถียร	ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
ผศ.ดร.สุรีย์ เจียรณ์มงคล	ภาควิชาเภสัชวิทยา คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ศ.นพ.ยง ภู่วรวรรณ	ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
ศ.นพ.เกียรติ รักษ์รุ่งธรรม	ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
รศ.นพ.ประสิทธิ์ ผลิตผลการพิมพ์	ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ
พญ.อรณี ตั้งเผ่า แดเนียล	*Executive Director, Medical Science* *Amgen Inc, Thousand Oaks,*
ศ.นพ.สุทัศน์ ฟู่เจริญ	สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยมหิดล
ผศ.นพ.เกื้อเกียรติ ประดิษฐ์พรศิลป์	ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ผศ.ดร.วัชรี ลิมปนสิทธิกุล	ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
รศ.นพ.ชัยชนะ นิ่มนวล	ภาควิชาจิตเวชศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

กำหนดการประชุมวิชาการประจำปี ครั้งที่ 30
สมาคมเภสัชวิทยาแห่งประเทศไทย
ร่วมกับ ภาคเภสัชวิทยา คณะแพทยศาสตร์ คณะเภสัชศาสตร์ คณะสัตวแพทยศาสตร์ คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

วันที่ 27-28 มีนาคม 2551

ณ ห้องประชุมสี สิริสิงห อาคารสมเด็จย่า ชั้น 2 คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

From Pharmacology to National Drug Policy

วันพฤหัสบดีที่ 27 มีนาคม 2551

9.00 - 8.45 น.	ลงทะเบียน
8.45 - 9.00 น.	ประธาน กล่าวรายงาน พิธีเปิดการประชุม โดย อธิการบดี จุฬาลงกรณ์มหาวิทยาลัย นายกสมาคมฯ กล่าวต้อนรับผู้เข้าร่วมการประชุม
10.15 - 10.30 น.	The 15th Dr.Chiravat Sadavongvivad Memorial Lecture: ยากับระบบประกันสุขภาพ วิทยากร : ศ.นพ.ธาดา ยิบอินซอย
10.15 - 10.30 น.	พัก- อาหารว่างและเครื่องดื่ม
10.30 - 12.00 น.	Symposium I: Rational drug use and update on essential drug list วิทยากร : ศ.พญ.สยมพร ศิรินาวิน และ ภญ.อรวรรณ เกตุเจริญ ผู้ดำเนินการอภิปราย : ผศ.นพ.พิสนธิ์ จงตระกูล
12.00 - 13.00 น.	Luncheon Symposium I : Blood pressure control and cardiovascular prevention : Evidence-based medicine 2008 ADVANCE : A step forward in Hypertension Treatment in type-2 Diabetes วิทยากร : อ.นพ.ยงเกษม วรเศรษฐการกิจ Advantages of Perindopril/Indapamide in patients with type-2 Diabetes วิทยากร : รศ.นพ.ดร.ศุภนิมิต ทีฆชุณหเถียร ผู้ดำเนินการอภิปราย : ผศ.ดร.สุรีย์ เจียรณ์มงคล
13.00 - 14.00 น.	Poster Session นำเสนอผลงานโดย: นิสิต/นักศึกษาบัณฑิตศึกษา พิจารณาผลงานโดย: คณะกรรมการพิจารณาผลงานวิจัย
14.00-15.45 น.	Symposium II : World wide viral infection and antiviral agents วิทยากร : ศ.นพ.ยง ภู่วรวรรณ ศ.นพ.เกียรติ รักษ์รุ่งธรรม ผู้ดำเนินการอภิปราย : รศ.นพ.ประสิทธิ์ ผลิตผลการพิมพ์

15.45-17.00 น.	ประชุมธุรการสมาคมฯ และเลือกผู้รั้งตำแหน่งนายกสมาคมฯ งานเลี้ยงต้อนรับผู้เข้าร่วมประชุม

วันศุกร์ที่ 29 มีนาคม 2551

8.30-9.45 น.	Plenary Lecture : Biosimilar : Concept in the quality assurance of generic biological products วิทยากร : พญ.อรณี ตั้งเผ่า แดเนียล
9.45-10.00 น.	พัก-อาหารว่างและเครื่องดื่ม
10.00-12.00 น.	Symposium III : Clinical experiences of biological products วิทยากร : ศ.นพ.สุทัศน์ ฟู่เจริญ ผศ.นพ.เกื้อเกียรติ ประดิษฐ์พรศิลป์ ผู้ดำเนินการอภิปราย : ผศ.ดร.วัชรี ลิมปนสิทธิกุล พญ.อรณี ตั้งเผ่า แดเนียล
12.00-13.00 น.	Luncheon Symposium II : New trend in the treatment of depression : Melatonin Agonist Serotonin Antagonist(MASA) : Unmet medical needs in the treatment of depression วิทยากร : รศ.นพ. ชัยชนะ นิ่มนวล : Circadian and Depression วิทยากร : ศ.นพ. อนันต์ ศรีเกียรติขจร New pharmacological approach for the treatment of depression วิทยากร : รศ.ดร. จินตนา สัตยาศัย ผู้ดำเนินการอภิปราย : รศ.ดร. ชัยชาญ แสงดี
13.00-15.00 น.	Symposium IV : Stem Cell : Facts and issues of concern วิทยากร : ศ.นพ.สุรพล อิสรไกรศีล อ.นพ.ภาคภูมิ เขียวละม้าย ผู้ดำเนินการอภิปราย: อ.นพ.ภาคภูมิ เขียวละม้าย
15.00-15.15 น.	พัก-อาหารว่างและเครื่องดื่ม
15.15-16.00 น.	ประกาศและมอบรางวัลการนำเสนอผลงานวิจัย นำเสนอผลงานโดยนิสิต/นักศึกษาผู้ได้รับรางวัล
16.00 น.	พิธีปิดการประชุมโดยนายกสมาคมฯ

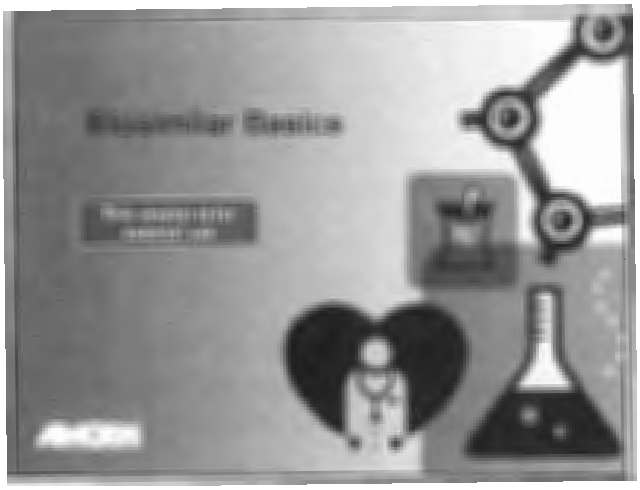
PL Biosimilars: General concepts

Oranee Tangphao MD

Executive Director, Medical Science
Amgen Inc, Thousand Oaks.

Abstract

Biosimilars, generic versions of large molecule biological products, has been a hot topic among healthcare and regulatory debates in recent years. The pressure for healthcare payors to contain the rising costs of healthcare, including pharmaceutical product cost, is a main driver for bringing less expensive drugs to the market. For generic versions of traditional small molecule pharmaceuticals, the standards for regulatory approval has been widely discussed and agreed upon since the 1980s. The criteria or requirements for traditional, generic small molecule drugs generally involve pharmaceutical equivalence and bioequivalence. These molecules are synthesized by chemical processes which usually yield relatively homogenous products. Most pharmacologists and regulatory reviewers are familiar with and have used these concepts although exceptions may be granted for certain drugs. In contrast, approval of biosimilars is likely to need a different set of criteria as biologics are synthesized or produced by living organisms. Several regulatory agencies around the world, especially EU and US are considering routes to evaluate and approve biosimilar products. Generally, biosimilar products should demonstrate comparability to the reference product in terms of 1) physicochemical properties, 2) biological activities, 3) its impurity profile and 4) clinical safety and efficacy. Due to the complexity of biologic manufacturing and the possibility of heterogeneity of the products, the necessity of biosimilars to prove clinical safety and efficacy in comparison to the reference product brings the burden of approval higher than with the generic version of traditional small molecule drugs. In addition, post-marketing surveillance will play an important role in assuring regulatory bodies and the public on the safety issues of these follow-on biologics. Details on biosimilar quality evaluation will be discussed during the presentation.



Amgen Guiding Principles

- Patients deserve safe and effective medicines
- Biosimilar medicines need careful evaluation by Regulatory bodies to ensure quality, safety, and efficacy
- Regulatory requirements should be transparent, science based, predictable and product specific
- Prescribing physicians should be fully informed about the Biosimilar medicines and actively involved in making a decision regarding substitution
- Innovator rights are fully respected

Context

Amgen is an innovative company with decades of experience in developing, manufacturing and marketing safe and efficacious Biotech medicines

In line with our values and as an industry leader, Amgen has been at the forefront of discussions on Biosimilars to ensure a science-based and transparent approach is followed for approval and use in medical practice

The release of draft EMEA guidelines, proposing requirements for approval of Biosimilars in four product-classes, has marked the beginning of a new phase of the Biosimilar discussion, accompanied by broader public awareness

As a science-based company, we will ensure that our staff has a well-balanced and fact-driven understanding of the nature and challenges of Biosimilars

Amgen's 1st priority is to ensure patient safety and continued well-being, and to use science and innovation to provide safe, breakthrough medical treatments

Agenda

Introduction

Definitions

Biosimilars vs. Generics

Regulatory Framework

Substitution

Agenda

Introduction

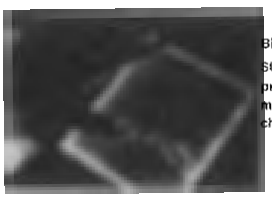
Definitions

Biosimilars vs. Generics

Regulatory Framework

Substitution

What Are Biotech Medicines?



Biotech medicines often replace or supplement a natural protein produced by the body, satisfying medical needs previously unmet by chemical medicines

More than 325 million patients worldwide have been helped by biotech medicines

Chemical Drugs vs. Biotech Medicines


Chemical drugs	Biotech medicines
Made by chemical synthesis	Made by living cells • Unique cell lines, from bacteria or mammals • Recombinant proteins
Defined structure and easy to characterize	Heterogeneous structure, difficult to characterize, mixtures of related molecules
Relatively stable	Variable; sensitive to conditions
Usually taken by mouth and prescribed by a general practitioner	Usually injected and prescribed by specialists

Terminology (1)

EU = Biosimilars

US = Follow-on Biologics (FOB)

But never-ever ~~Biogenerics~~

What Are Biosimilars?

The expiry of patent protection for certain Biotech medicines has led to the development of what are called Biosimilars

Biosimilars attempt to copy the **original innovative biotech medicine**

However, Biosimilars are actually different products, not accurate copies

Do Biosimilars Already Exist?

So far, no Biosimilars authorized at EU level or in the US, but ...

... Multiple Epoetins and G-CSFs under development

... Dossiers for HGH and Interferons have been submitted and are currently being reviewed in the EU

... There are marketed biological products from non-innovator companies outside the EU and US

Intense interest in US and EU in developing a regulatory pathway for approval

Characteristics Of Biosimilars

Biosimilars ...

... Are biological products that claim to be similar to an innovator biological product

... The innovator's product is off-patent and no regulatory data protection remains

... Are manufactured by a second manufacturer with new cell line, new process and new analytical methods

... Require original data for approval

Summary - Definitions

1. **Biotechnology medicines are:**
   - **Made by living cells**
   - **Replace or supplement a protein**

2. **Biosimilars attempt to copy the original innovative biotechnology medicine, but they are not identical**

3. **Biotech medicines cannot be copied because they are derived from unique living cells and organisms**

Biosimilars are only similar – not identical

Agenda

- Introduction
- Definitions
- Biosimilars vs. Generics
- Regulatory Framework
- Substitution

Molecular Properties

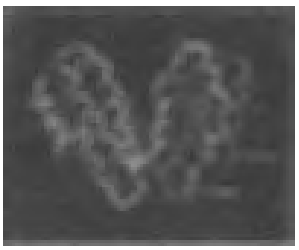
Biotech medicines are more complex

- Large numbers of molecular components
- Bigger molecular size
- Lack of exacting chemical specifications
- Mode of action is very complicated

Terminology (2)

Chemical drugs	Biotech Medicines
copy	attempt to copy
"Generics"	Biosimilars
	Follow-on Biologics

Example: Interferon Beta vs. Aspirin



Interferon Beta
MW 19'000D

Aspirin:
MW 180D

Four Things To Keep In Mind To Differentiate Biosimilars And Generics

- Molecular Properties
- Manufacturing Process
- Safety
- Efficacy

Protein Structure: Building A Biotech Medicine

Primary:
Amino Acid Sequence

Secondary:
Interaction of Amino Acids

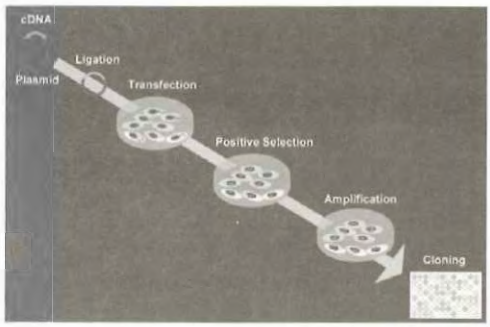
Tertiary:
Hydrophobic and Hydrophilic Interactions, Disulfide Bonds, and Posttranslational Modifications

Quaternary:
Interactions With Other Proteins

Summary – Molecular Properties

- Biosimilars are more complex than Generics
- Manufacturing Process
- Safety
- Efficacy

Each Manufacturer Must Make Their Own Unique Cell Line



Manufacturing: The Process Makes The Product

A highly controlled manufacturing process is intrinsically important to biotech medicines ...

... because control equals consistency

Cell Line Construction is Complex

- Clone to single cell
- Evaluate and compare clones
  - Growth
  - Viability
  - Productivity
  - Protein integrity
  - Genetic stability
- Bank

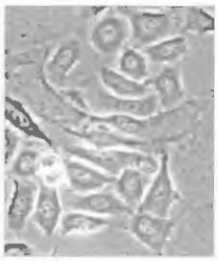
Characteristics Of the Manufacturing Process

- Takes months, not weeks, to produce a run
- Cost per run can be millions of euros
- To obtain consistent results, precise controlling necessary

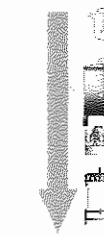
Manufacturing biotech medicines is complex, lengthy and expensive

Each Manufacturer's Cell Line is Unique

- Cell line is descended constructed using a unique proprietary DNA expression vector
- The characteristics of the incorporation of the DNA is unique for each cell
- The cell line is evaluated for product integrity, activity and overall quality
- In addition to product quality, the cell line is chosen based on expected performance in manufacturing such as growth and viability



Main Manufacturing Process Steps



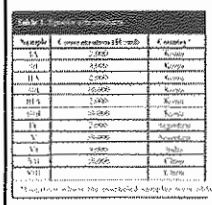
Unit Operation	Specific to Product
Cell Expansion	Cell line, growth media, method of expansion
Cell Production in Bioreactors	Cell line, growth media, bioreactor conditions
Recover through filtration or centrifugation	Operating conditions
Purification through chromatography	Binding and elution conditions
Characterization and Stability	Methods, reagents, reference standards

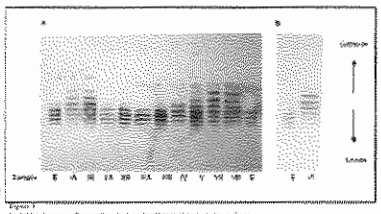
Purified Bulk Drug

Safety And Efficacy: Dogma Of Generics Does Not Apply To Biosimilars

Generics	✓ Bioavailability	⇒	✓ Safety ✓ Efficacy
Biosimilars	✓ Bioavailability	⇒	✗ Safety ✗ Efficacy

Example: Different EPO Alfas Can Vary Widely





Due to the complexity of molecule and manufacturing process, Biosimilars are never identical to originator products

Safety Considerations

For Biotech Medicines, each product has a unique safety profile dependent on its

- Mechanism of action
- Unique manufacturing process
- Composition (by-products and impurities)

Consequently, Biosimilar medicines need to have pre-approval safety data in addition to robust pharmacovigilance

Summary – Manufacturing Process

Molecular Properties

Manufacturing process is core to the product

- Extremely sensitive to changes in manufacturing and production
- Minor variations could produce vastly different products

Safety

Efficacy

Immunogenicity – A Unique Safety Issue For Biotech Medicines And Biosimilars

Therapeutic proteins have the potential to induce antibody responses

- Initial response to therapeutic protein
- Later broad response incl. endogenous protein

In many cases, no clinical consequences but antibody-related reaction can be serious, even life-threatening

Immunogenicity of Biosimilars cannot be predicted with preclinical or non-human studies

- Clinical studies required pre-approval
- Robust pharmacovigilance needed

Summary - Safety

Molecular Properties

Manufacturing Process

- Safety profile of Biosimilars is unpredictable
- Prescribers and patients must be fully aware of the potential issues

Efficacy

Agenda

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Manufacturing Changes Can Impact Pharmacokinetics and Efficacy

Manufacturing site for Raptiva was transferred from Xoma to Genentech

- No differences in analytical characterization data between the Xoma product and the Genentech product
- But: products did not demonstrate bioequivalence
- An additional phase III study was carried out to support dosing, efficacy, and safety
- The preparation with higher peripheral drug concentration demonstrated a trend towards a lower clinical response

Pharmacokinetic data can be unpredictable with regard to clinical response

Regulatory Status In The EU

Unlike the US, the EU is drafting guidance on appropriate pathways for the clinical approval of Biosimilars

- The EU legislation is the first to create an abbreviated legal pathway for approving Biosimilars
- European regulators are now deciding on the process to bring Biosimilars to market

The European Medicines Agency is currently collecting data from stakeholders on non-clincal and clinical guideline and on four product specific guidelines to manufacture Biosimilars

Summary - Efficacy

Molecular Properties

Manufacturing Process

Safety

Efficacy can differ significantly with small changes in protein or formulation

Overview Of Planned EMEA Guidelines

Likely to be adopted in 1Q 2006

TOPIC	TITLE	APPLICATION
Overarching	Guideline on Similar Biological Medicinal Products	General: Applies to all Biosimilars
Quality	Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Quality Issues	
Nonclinical & Clinical	Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Nonclinical & Clinical Issues	
Annexes Nonclinical & Clinical	Recombinant Human Erythropoietin; Recombinant Human G-CSF; Recombinant Human Insulin; Recombinant Human Growth Hormone	Specific: Product data requirements

Key Points Of Draft G-CSF Guidelines

	EMEA Draft Guidance
Preclinical	Comparative non-clinical studies 28-day toxicology
Human PK equivalence	Single dose in healthy volunteers using SC and IV
Efficacy	2-arm equivalence trial in CIN setting of known neutropenic suppression. *OR* PD study in healthy volunteers (if justified)
Extrapolation	Yes – Equivalence in CIN can extrapolate to other indications if mechanism of action is the same
Safety	6-months to evaluate AE's and immunogenicity

Agenda

- Introduction
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Key Points Of Draft EPO Guidelines

	EMEA Draft Guidance
Preclinical	Comparative non-clinical studies 3-month toxicology
Human PK equivalence	Single dose in healthy volunteers using SC and IV
Efficacy	2, double blind studies in nephrology SC for predialysis and IV for haemodialysis Dose and Hb levels to be collected
Extrapolation	Yes – data in nephrology may allow extension to other indications if same mode of action
Safety	300 patients from efficacy trial 1-year immunogenicity data
Pharmacovigilance	No precise requirements but immunogenicity identified as topic to be addressed

Why Do We Discuss Substitution?

Substitution

raises

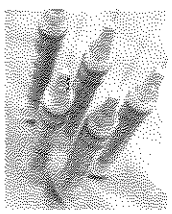
concerns for Patient Safety

Summary – Regulatory Framework

EMEA product-specific guidelines still draft, final version expected for Q1 2006

Amgen's comments to EMEA focus on:

- Pre-approval safety database
- Extrapolation to different indications
- Pharmacovigilance requirements



Terminology

Generic = Identical

Epoetin alfa ⟺ Biosimilar Epoetin alfa

Therapeutic substitution

Darbepoetin alfa ⟺ Epoetin alfa

What Is The Principle Of Substitution?

Medicines are the same = therefore can be substituted

✓ Generics / chemical drugs

Substances are identical = therefore can be substituted

For Biosimilars, Substitution Would Carry An Even Greater Risk For The Patient

- Biosimilars do not have the same active ingredient as the innovator
- Biosimilars may not have same safety & efficacy profile as the innovator
- Conventional bioequivalence studies alone cannot assure clinical safety and efficacy of biotech medicines

Automatic substitution of the original treatment with a Biosimilar represents a safety risk since the consequences of such substitution are unpredictable

- Limited safety database in populations
- Patient dose titration and inter-patient variation
- Immunogenicity

The physician should be responsible for providing the best treatment to the patient

Principle Of Generic Substitution Does Not Apply To Biosimilars

Medicines are the same = therefore can be substituted

✓ Generics / chemical drugs

Substances are identical = therefore can be substituted

X Biosimilars/Biotech medicines

Are not identical = therefore generic substitution not possible

Physicians And Patients Need To Be Involved In Decisions On Substitution

Physicians should be provided full details regarding any biotech medicine in original labeling for each product

- Unique clinical data
- Limited safety data for Biosimilars
- Warnings regarding immunogenicity and substitution without physician involvement

Products need to have unique identification

- Promote safe use and prescription
- Promote accurate data reporting and pharmacovigilance
- Ensure timely and accurate actions in the postmarketing setting
  - INN
  - Brand name

Even For Small Molecules,Substitution Carries A Risk

Loss of seizure control and drug related toxicity following substitution with anticonvulsants

Significant differences in pharmacokinetic parameters and patient variability between generic and brand versions of antiarrythmic procainamide leading to differential suppression of ventricular ectopy and emergence of arrhythmias; after switching

Differences in safety profile among generic hormone preparations

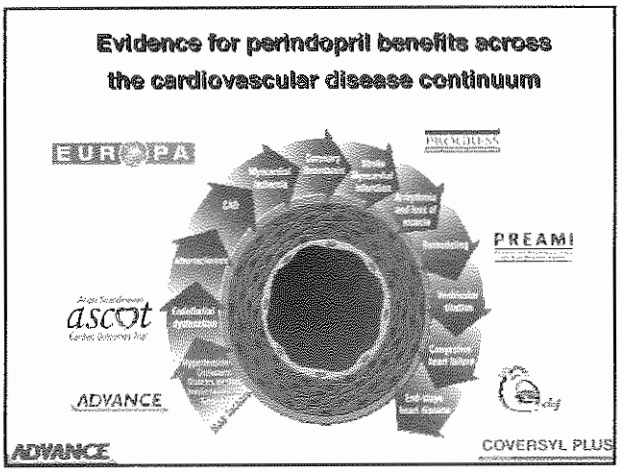
Withdrawal of generic cyclosporine A due to differences in absorption from the branded version that was not identified by conventional bioequivalence studies

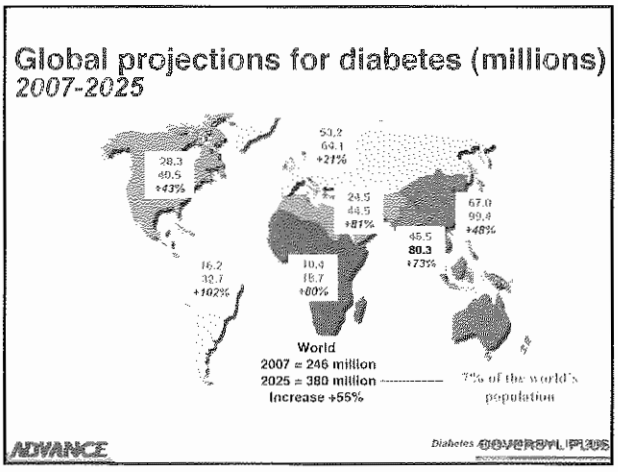
Summary - Substitution

Biosimilars are not identical to originator, therefore generic substitution rules do not apply

Physician needs to be involved in substitution decisions

- Risk of immunogenicity
- Patient dose titration and inter-patient variation
- Pre-exposure to other biological therapies


Evidence for perindopril benefits across the cardiovascular disease continuum
EUROPA
PROGRESS
PREAMI
ascot
ADVANCE
ADVANCE
COVERSYL PLUS


Global projections for diabetes (millions)
2007-2025
28.3
40.5
+43%
50.2
64.1
+21%
24.5
44.5
+81%
10.4
18.7
+80%
16.2
32.7
+102%
45.5
80.3
+73%
67.0
99.4
+48%
World
2007 = 246 million
2025 = 380 million
Increase +55%
7% of the world's population
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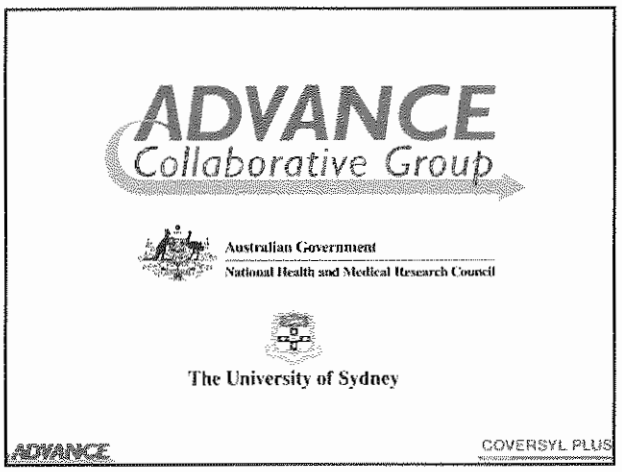
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Collaborative Group
Australian Government
National Health and Medical Research Council
The University of Sydney
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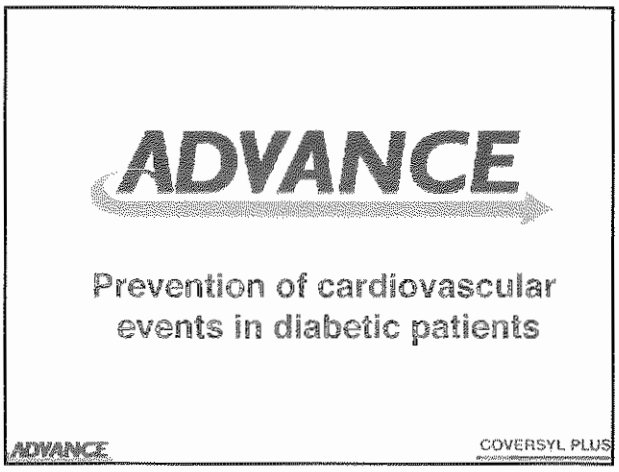
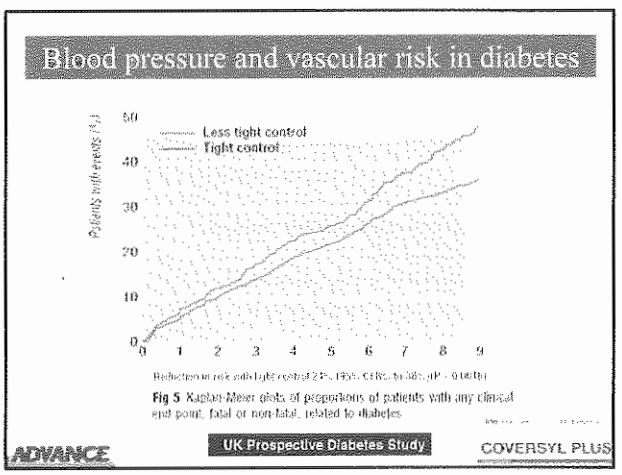
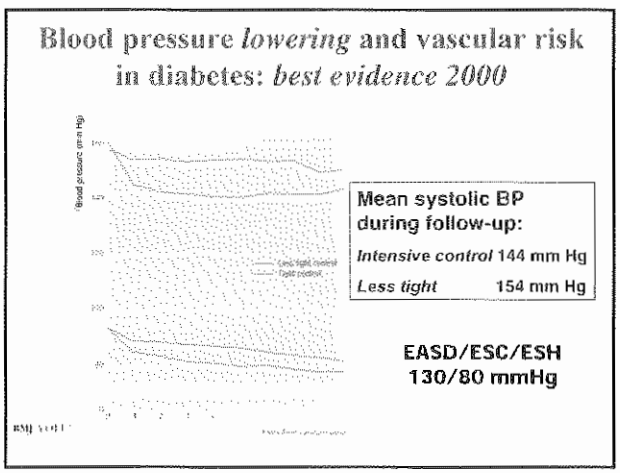

ADVANCE
Prevention of cardiovascular events in diabetic patients
ADVANCE
COVERSYL PLUS


Blood pressure and vascular risk in diabetes
Less tight control
Tight control
0
10
20
30
40
50
0 1 2 3 4 5 6 7 8 9
Fig 5 Kaplan-Meier plots of proportions of patients with any clinical end point, fatal or non-fatal, related to diabetes
UK Prospective Diabetes Study
ADVANCE
COVERSYL PLUS


Blood pressure lowering and vascular risk in diabetes: best evidence 2000
Mean systolic BP during follow-up:
Intensive control 144 mm Hg
Less tight 154 mm Hg
EASD/ESC/ESH
130/80 mmHg

Blood pressure lowering in diabetes: Unresolved issues 2000

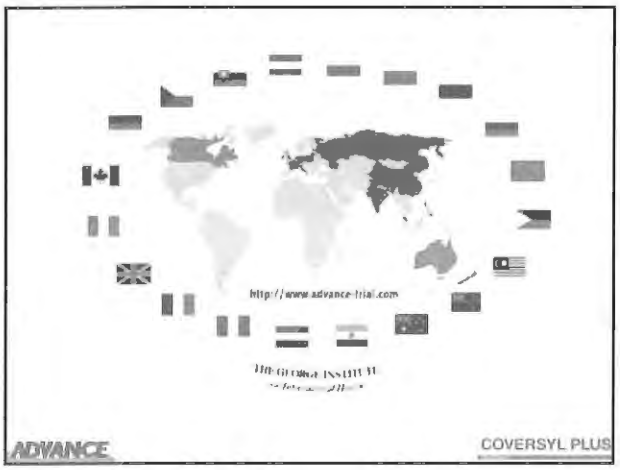
- *Among patients with diabetes, does blood pressure lowering therapy:*
  - Produce additional benefits when systolic pressure is lowered below 145 mmHg?
  - Produce similar benefits for hypertensive and non-hypertensive patients?
  - Add to the benefits produced by other cardiovascular preventive therapies including ACE inhibitors?

ADVANCE COVERSYL PLUS

ADVANCE study hypotheses
Perindopril-indapamide arm

- *Among patients with diabetes, does blood pressure lowering therapy:*
  - Produce additional benefits when systolic pressure is lowered below 145 mmHg?
  - Produce similar benefits for hypertensive and non-hypertensive patients?
  - Add to the benefits produced by other cardiovascular preventive therapies including ACE inhibitors?

ADVANCE COVERSYL PLUS



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Inclusion criteria

- Type 2 diabetes mellitus
- Age 55 years or older
- Additional risk of vascular event
  - Age ≥ 65 years
  - History of major macrovascular disease
  - History of major microvascular disease
  - First diagnosis of diabetes >10 years prior to entry
  - Other major risk factor
- Hypertensive or normotensive

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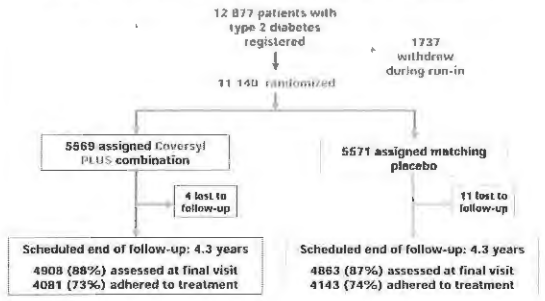
ADVANCE *trial profile*



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Baseline characteristics

	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
Age (y)	66	66
Systolic blood pressure (mm Hg)	145	145
Diastolic blood pressure (mm Hg)	81	81
Hemoglobin A_{1c} (%)	7.5	7.5
History of macrovascular disease (%)	32	32
History of microvascular disease (%)	10	10
Microalbuminuria (%)	26	26

ADVANCE COVERSYL PLUS

Baseline characteristics
Cardiovascular and diabetes

	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
Any blood pressure-lowering drugs	75%	75%
ACE inhibitors*	43%	43%
Oral hypoglycemic drugs	91%	91%
Statins	28%	29%
Other lipid-modifying drugs	9%	8%
Aspirin	44%	44%
Other antiplatelet drugs	4%	5%

By the end of the run-in period, 42% of patients were receiving open-label perindopril

ADVANCE COVERSYL PLUS

Withdrawals during run-in

Reason for withdrawal	N	Registered patients (N=12 877)
Patient ineligible	394	3.1%
Patient wishes	391	3.0%
Poor compliance with study drug	269	2.1%
Cough	238	1.8%
Hypotension	99	0.8%
Other suspected intolerance to study drug	133	1.0%
Other reasons	213	1.7%
TOTAL	1737	13.5%

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Primary study outcomes

Combined macrovascular and microvascular end points

Macrovascular

Death from any cardiovascular cause, or nonfatal stroke, or nonfatal myocardial infarction

Microvascular

New or worsening nephropathy (microalbuminuria), or diabetic eye disease

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Results

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ADVANCE

Follow-up and adherence

ADVANCE COVERSYL PLUS

Reasons for discontinuation

Major reasons for discontinuation	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
Patient unable/unwilling to attend visits	521 (9.4%)	635 (11.4%)
Cough	184 (3.3%)	72 (1.3%)
Hypotension or dizziness	69 (1.2%)	22 (0.4%)
Serious adverse events	67 (1.2%)	66 (1.2%)
Other reasons	172 (3.1%)	195(3.5%)

ADVANCE COVERSYL PLUS

Adherence to study treatments

Follow-up visits *(months)*	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
12	4950 (89%)	5081 (91%)
24	4676 (84%)	4776 (86%)
36	4403 (79%)	4518 (81%)
48	4164 (75%)	4287 (77%)
Final visit	4081 (73%)	4143 (74%)

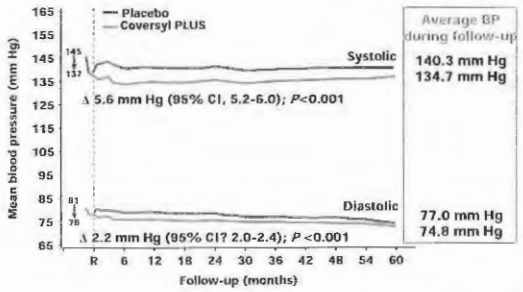
ADVANCE COVERSYL PLUS

ADVANCE

Blood pressure, other risk factors, and ancillary treatment

ADVANCE COVERSYL PLUS

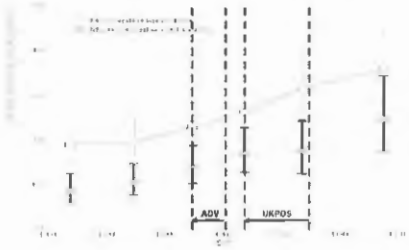
Blood pressure reduction



ADVANCE COVERSYL PLUS

ADVANCE in context:

UK Prospective Diabetes Study



ADVANCE goes beyond UKPDS

Risk factor levels

At end of follow-up

Parameter	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
Systolic BP (mm Hg)	135.6	139.9
Diastolic BP (mm Hg)	73.6	75.1
Hemoglobin A_{1c} (%)	6.9	6.9
Total cholesterol (mmol/L) *	4.7	4.6
HDL cholesterol (mmol/L) *	1.3	1.3
LDL cholesterol (mmol/L) *	2.7	2.6
Triglycerides (mmol/L) *	1.8	1.7

*Measurements taken at 48 months

ADVANCE COVERSYL PLUS

Ancillary drug therapy

At end of follow-up

	Randomized treatment	
	Active (n=5569)	Placebo (n=5571)
Any BP-lowering drug	74%	83%
ACE inhibitors	50%	60%
Oral hypoglycemic drugs	90%	91%
Insulin	33%	30%
Statins	44%	45%
Other lipid-modifying drugs	8%	7%
Aspirin	56%	55%
Other antiplatelet drugs	6%	6%

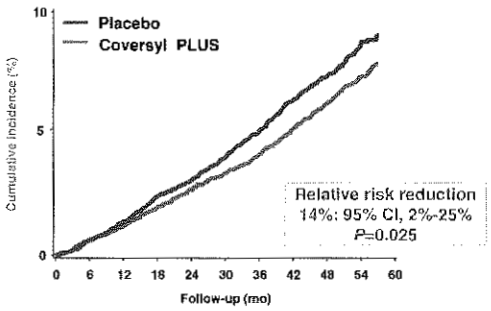
ADVANCE COVERSYL PLUS

ADVANCE

Mortality and morbidity

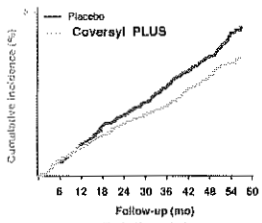
ADVANCE COVERSYL PLUS

Coversyl PLUS decreases all-cause mortality



ADVANCE UKPDS & Micro-HOPE no benefits?! COVERSYL PLUS

Coversyl PLUS decreases cardiovascular mortality



Relative risk reduction 18%; *P*=0.027

ADVANCE COVERSYL PLUS

Coversyl PLUS decreases coronary events

	N events Cov PLUS (n=5569)	Placebo (n=5571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
All coronary heart disease	468	535			14% (2 to 24)*
Major coronary heart disease†	265	294			11% (-8 to 24)
Other coronary heart disease‡	283	324			14% (-1 to 27)

0.5 1.0 2.0
Hazard ratio

*P=0.02
†Nonfatal MI, or death from coronary heart disease
‡Unstable angina requiring hospitalization, coronary revascularization, or silent MI

ADVANCE COVERSYL PLUS

Coversyl PLUS decreases renal events

	N events Cov PLUS (n=5569)	Placebo (n=5571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
Total renal events	1243	1500			21% (15 to 27)*
New or worsening nephropathy	181	216			18% (-1 to 32)
New microalbuminuria	1094	1317			21% (14 to 27)

0.5 1.0 2.0
Hazard ratio

*P=<0.01

ADVANCE COVERSYL PLUS

Absolute benefits of routine treatment with Coversyl PLUS

After 5 years, treatment would prevent:	Among every
1 major vascular event	66 patients
1 death	79 patients
1 coronary event	75 patients
1 renal event*	20 patients

Mostly new-onset microalbuminuria

ADVANCE COVERSYL PLUS

ADVANCE

Subgroups

Combined primary outcome

ADVANCE COVERSYL PLUS

Effects by age, sex, BP, and HbA_{1c}

Combined primary end point

	N events Cov PLUS (n=5569)	Placebo (n=5571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
Age (y)					
< 65	325	346			6% (-10% to 19%)
≥ 65	536	592			11% (0% to 21%)
Sex					
Male	546	594			10% (-1% to 20%)
Female	315	344			8% (-7% to 21%)
SBP (mm Hg)					
< 140	309	341			10% (-5% to 23%)
≥ 140	552	597			9% (-2% to 19%)
History of hypertension					
No	121	136			9% (-17% to 29%)
Yes	740	802			9% (0% to 18%)
HbA_{1c} (%)					
≤ 7.5	406	456			9% (-4% to 20%)
> 7.5	451	481			11% (-1% to 22%)
All participants	861	938			9% (0% to 17%)

0.5 1.0 2.0
Hazard ratio

P heterogeneity all >0.1

ADVANCE COVERSYL PLUS

Effects by ancillary treatment

Combined primary endpoint

	N events Cov PLUS (n=5,569)	Placebo (n=5,571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
Treatment with any BP-lowering drug					
No	177	183			6% (-15% to 24%)
Yes	684	755			10% (0% to 19%)
Treatment with ACE inhibitors					
No	417	455			10% (-3% to 21%)
Yes	444	483			8% (-4% to 20%)
Treatment with statins					
No	638	687			10% (0% to 19%)
Yes	223	251			8% (-10% to 23%)
Treatment with antiplatelet drugs					
No	408	454			11% (-2% to 22%)
Yes	453	484			7% (-5% to 18%)
All participants	861	938			9% (0% to 17%)

0.5 1.0 2.0
Hazard ratio

P heterogeneity all >0.1

ADVANCE COVERSYL PLUS

ADVANCE

Subgroups

New-onset microalbuminuria

ADVANCE COVERSYL PLUS

Effects by age, sex, BP, and HbA_{1c}

Microalbuminuria

	N events Cov PLUS (n=5589)	Placebo (n=5571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
Age (y)					
< 65	448	522			16% (5% to 26%)
≥ 65	646	795			24% (15% to 31%)
Sex					
Male	661	724			21% (12% to 29%)
Female	493	593			20% (9% to 29%)
SBP (mm Hg)					
< 140	455	535			18% (4% to 29%)
≥ 140	629	782			24% (16% to 32%)
History of hypertension					
No	178	218			19% (1% to 34%)
Yes	916	1099			21% (14% to 28%)
HbA_{1c} (%)					
≤ 7.5	640	795			20% (11% to 28%)
> 7.5	444	517			22% (11% to 31%)
All participants	1094	1317			21% (14 to 27)

0.5 1.0 2.0
Hazard ratio

P heterogeneity all >0.1

ADVANCE COVERSYL PLUS

Effects by ancillary treatment

Microalbuminuria: effect of 8 mg/d of perindopril

	N events Cov PLUS (n=5569)	Placebo (n=5571)	Favors Cov PLUS	Favors Placebo	Relative risk reduction (95% CI)
Treatment with any BP-lowering drug					
No	254	312			21% (7 to 33)
Yes	830	1005			20% (13 to 27)
Treatment with open-label perindopril					
No	588	671			16% (6 to 25)
Yes	506	646			**25% (16 to 33)**
Treatment with statins					
No	816	987			23% (15 to 30)
Yes	278	330			15% (1 to 28)
Treatment with antiplatelet drugs					
No	568	697			22% (13 to 30)
Yes	526	620			19% (9 to 28)
All participants	1094	1317			21% (14 to 27)

0.5 1.0 2.0
Hazard ratio

P heterogeneity all >0.1

ADVANCE COVERSYL PLUS

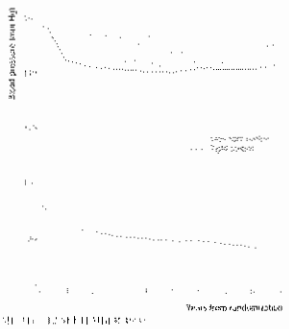
Summary

- **Routine treatment of type 2 diabetic patients with Coversyl PLUS resulted in:**
  - 14% reduction in total mortality
  - 18% reduction in cardiovascular death
  - 9% reduction in major vascular events
  - 14% reduction in total coronary events
  - 21% reduction in total renal events

Similar benefits in all major subgroups.
Benefits additional to those produced by other treatments, including ACE inhibitor.
Treatment very well-tolerated.

ADVANCE COVERSYL PLUS

Blood pressure *lowering* and vascular risk in diabetes: *best evidence 2000*

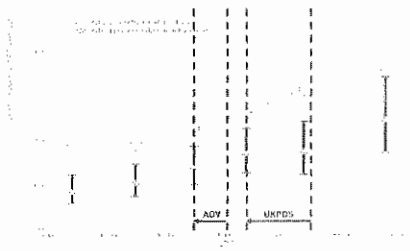


Mean systolic BP during follow-up:
Intensive control 144 mm Hg
Less tight 154 mm Hg

ADVANCE UK Prospective Diabetes Study COVERSYL PLUS

ADVANCE in context:

UK Prospective Diabetes Study



ADVANCE goes beyond UKPDS

BMJ. 2000;321.
ADVANCE COVERSYL PLUS

Blood pressure lowering in diabetes: *unresolved issues 2000*

Among patients with diabetes, does blood pressure-lowering therapy:

1. Produce additional benefits when systolic blood pressure is lowered below 145 mm Hg? YES
2. Produce similar benefits for hypertensive and nonhypertensive patients? YES
3. Produce benefits on top of other treatments, including ACE inhibitors and other antihypertensive drugs? YES

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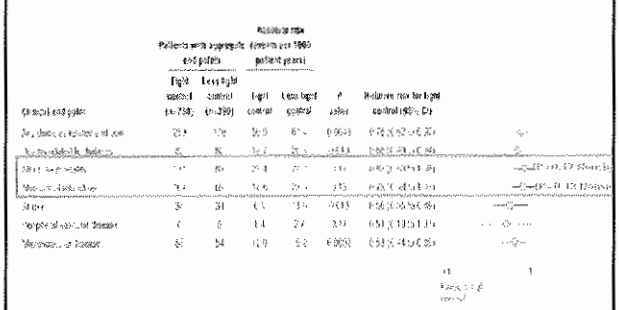
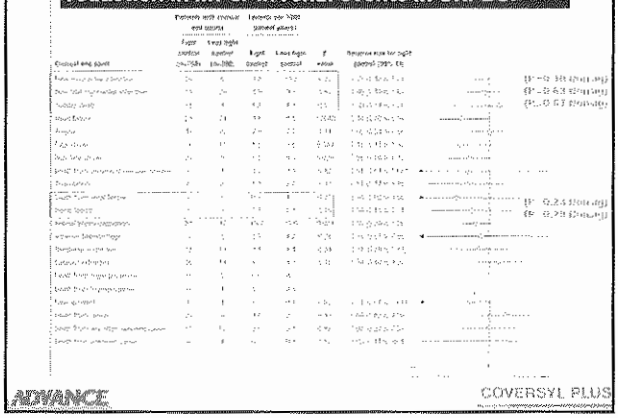
But difference in BP does not provide significant reduction in all cause death and MI in UKPDS (p=0.17, MI p=0.13)



UKPDS 38 results

ADVANCE COVERSYL PLUS

But difference in BP does not provide significant reduction in MI or in renal events in UKPDS!



ADVANCE COVERSYL PLUS

ADVANCE in context:

Comparative patient profiles: UKPDS, MicroHOPE, ADVANCE

	UKPDS (n=1148)	MicroHOPE (n=3577)	ADVANCE (n=11148)
BP levels (mm Hg) Active treatment at the end of follow-up	145/82	139/77	**136/73**
Use of a background ACE inhibitor	No	No	Yes
Use of statins	No	+	+++
HbA_{1c} at the end of follow-up	8.3%	9.5%	**6.9%**
Event rates			
Total mortality and CV mortality	+++	+++	++
Stroke	++	+++	+
Total mortality reduction	no	no	-14% (p<0.025)

Remarks : COVERSYL PLUS very effective even in a low risk patients from ADVANCE Trial COVERSYL PLUS

Absolute benefits of routine treatment with Coversyl PLUS

After 5 years, treatment would prevent:	Among every
1 major vascular event	66 patients
1 death	79 patients
1 coronary event	75 patients
1 renal event*	20 patients

*Mostly new-onset microalbuminuria

ADVANCE COVERSYL PLUS

Potential global benefits of treatment *2010-2015*

If the benefits observed in ADVANCE were applied to just half of the world's diabetic population, approximately 1.5 million deaths could be avoided over this period

If the benefits observed in ADVANCE were applied to just half of the Thai diabetic population,approximately 1 deaths for every 2 hours could be avoided. (1000/year)

ADVANCE *Diabetes Atlas*, 3rd edition, IDF 2006 COVERSYL PLUS

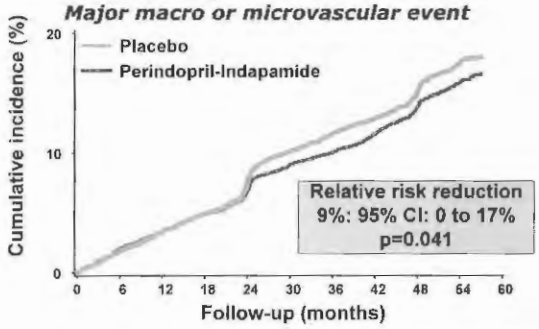
Advantages of Perindopril/Indapamide in patients with type 2 diabetes

ดร.นพ.สุภนิมิต ทีฆชุณหเถียร
รองศาสตราจารย์ ภาควิชาเภสัชวิทยา
คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

ADVANCE

Combined primary outcomes

Major macro or microvascular event



Lancet 2007;370:829-40.

Advantages of Per/Ind in patients with type 2 diabetes

BP-lowering effect

Advantages of Per/Ind in patients with type 2 diabetes

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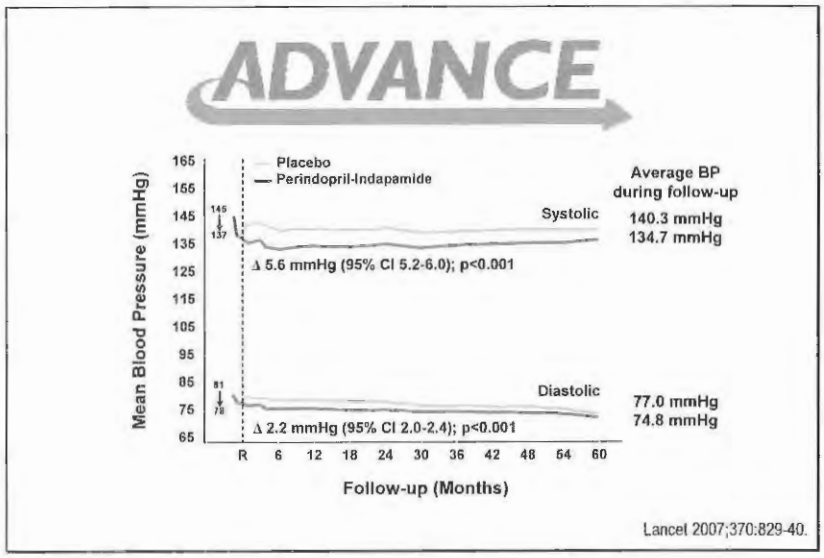
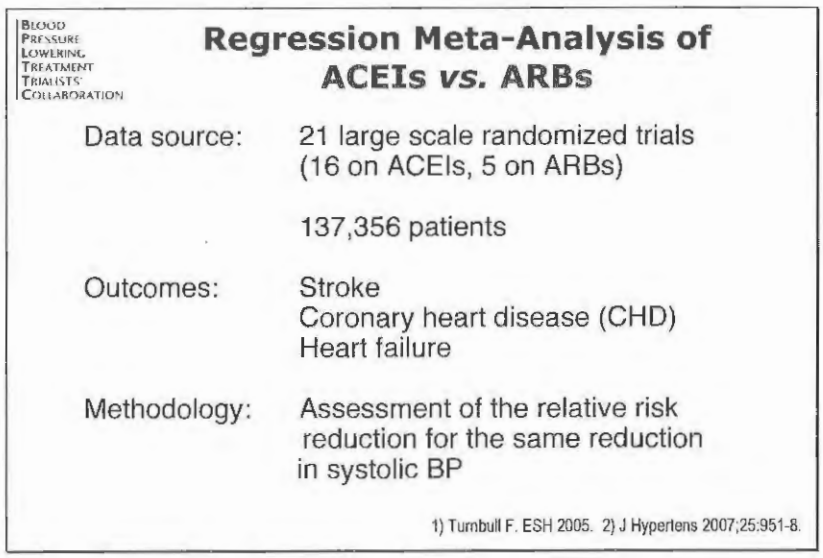
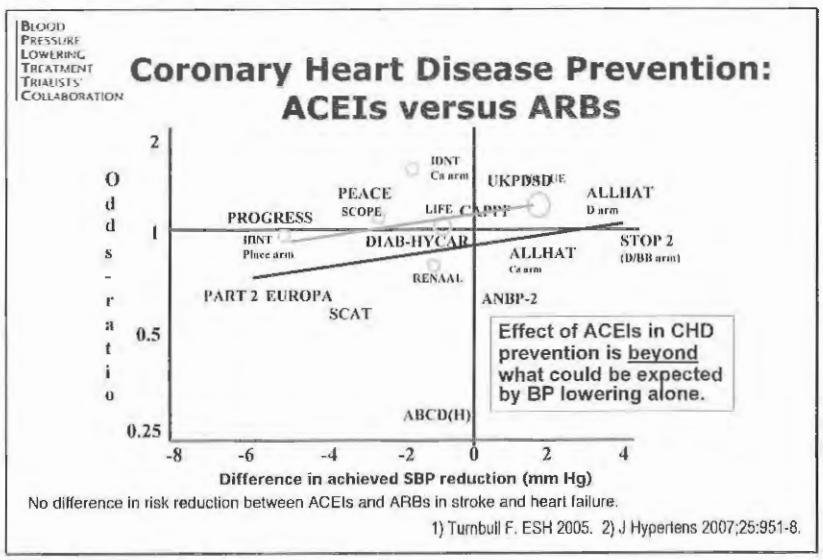


Lancet 2007;370:829-40.





Advantages of Per/Ind in patients with type 2 diabetes

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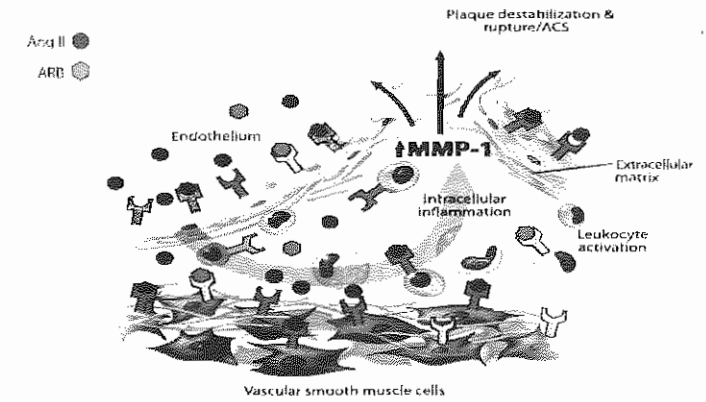
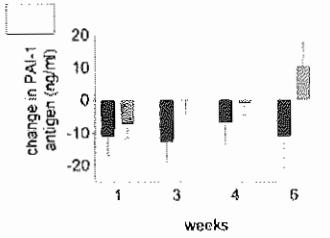
Role of AT2 receptor in Plaque Rupture



J Leukoc Biol 2005;78:195-201.

Effects of ACEIs vs ARBs on PAI-1 Expression

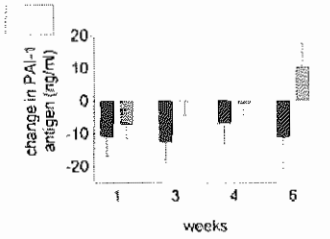


Change in PAI-1 over time in response to treatment with ramipril (ACEI) or losartan (AT_1RA) in patients with essential hypertension and insulin resistance taking HCTZ[1].

- **ACEIs potentiate the effects of systemic bradykinin, a stimulus to endothelial NO release.**
- **Recent studies indicate that NO suppresses PAI-1 expression after stimulation by Ang II in aortic smooth muscle cells[2].**

[1]Hypertension 2002;40:859-65.
[2] Arterioscler Thromb Vasc Biol 1998;18:1771–9.

Effects of ACEIs vs ARBs on PAI-1 Expression



Change in PAI-1 over time in response to treatment with ramipril (ACEI) or losartan (AT_1RA) in patients with essential hypertension and insulin resistance taking HCTZ[1].

- **Ang II possibly increases PAI-1 expression in humans through its hexapeptide metabolite, Ang IV, and the Ang II type 4 receptor (AT_4), which has been observed in vitro in endothelial cells[2].**

[1]Hypertension 2002;40:859-65.
[2]J Clin Invest 1995;96:2515-20.

Advantages of Per/Ind in patients with type 2 diabetes

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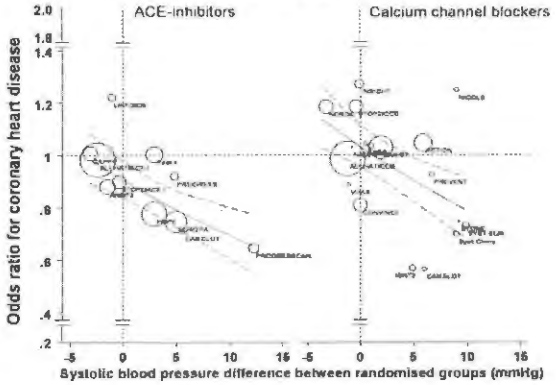
Coronary Heart Disease Prevention: ACEIs versus CCBs

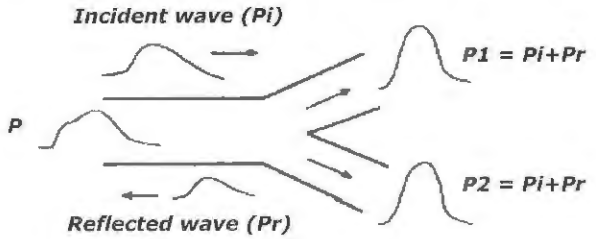


Hypertension 2005;46:386-392.

Advantages of Per/Ind in patients with type 2 diabetes

2 Reduced pulse wave reflection

Genesis of aortic pressure waveform



- The incident pressure wave in an artery, transmitted from the central aorta, is reflected back (wave reflection) and amplified toward the periphery at any point of impedance discontinuity, such as arterial branching and arterial-arteriolar junctions.

Circ J 2006;70:1231-9.

Advantages of Per/Ind in patients with type 2 diabetes

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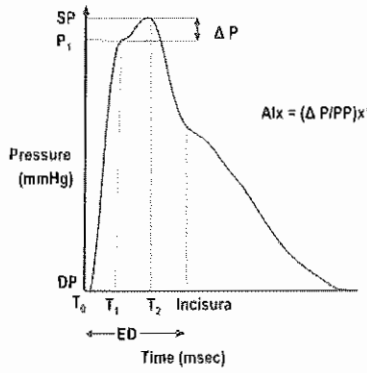
Pulse wave analysis of the central aortic pressure wave



T0 indicates the time at the start of the waveform;
T1, duration from start of waveform to the first peak/shoulder (outgoing pressure wave);
T2, duration from start of waveform to the second peak/shoulder (reflected pressure wave);
ED, ejection duration, or duration from start of waveform to closure of the aortic valve (incisura);
SP, central aortic systolic pressure;
DP, central aortic diastolic pressure;
P1, P1 height difference between the minimum pressure and the pressure at the first peak/shoulder (T1);
Augmentation (P), difference between maximal pressure (central aortic systolic pressure) and pressure at the fist peak/shoulder (P1 height);
PP, pulse pressure;
AIx, augmentation index.

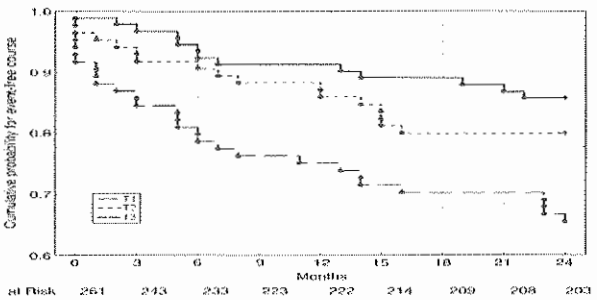
Circulation 2006;113:1213-25.

Importance of aortic pressure wave

- The most physiologically relevant pressures for both cardiac and vascular effects are central pressures.
- Central systolic pressure is the pressure that the left ventricle has to confront.
- Central pulse pressure is a better predictor of left ventricular mass and carotid–intima thickness than peripheral pulse pressure.

Hypertension 2001;38:1456–60.
AJH 2006;19:214–9.

Kaplan–Meier estimates of the rates of the primary endpoint* according AIx@75



262 patients undergoing PCI, 2-year follow-up.
**Death, MI, and clinical restenosis.*

Eur Heart J 2005; 26: 2657-63.

Advantages of Per/Ind in patients with type 2 diabetes

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REASON: Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind Study

- TRIAL DESIGN -

Design

Multicenter, controlled, randomized, double-blind, 2-parallel groups study conducted in 13 countries

Patients

562 patients with essential hypertension

Follow up and primary endpoint

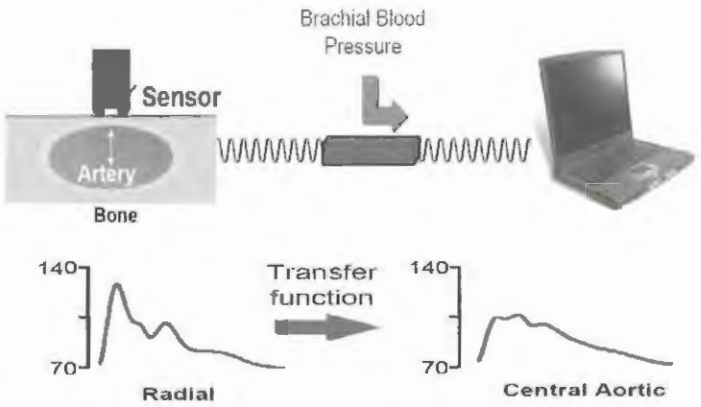
Brachial BP, aortic or carotid pulse wave analysis, pulse wave velocity (PWV), heart rate. 12-month active treatment period.

Treatment

Perindopril/Indapamide (2 mg/0.625 mg) or atenolol (50 mg), and the dose was doubled after 3 months if SBP remained >160 mmHg and/or DBP >90 mmHg.

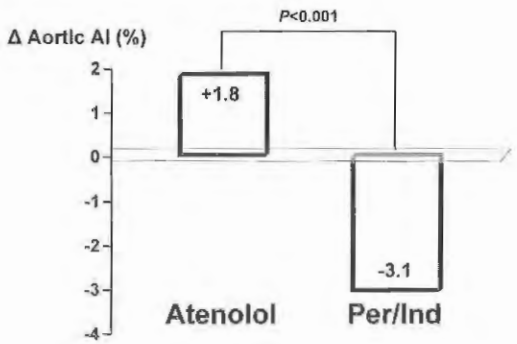
Hypertension 2001;38:922–6.

Pulse wave analysis



REASON: Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind Study

- RESULTS -



Hypertension 2001;38:922–6.

Advantages of Per/Ind in patients with type 2 diabetes

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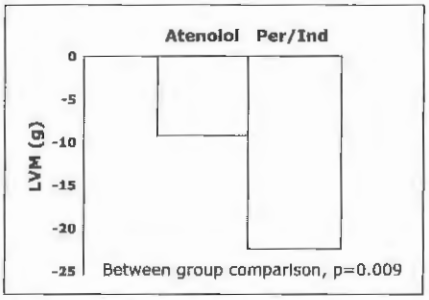
REASON: Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind Study

- The differences in central blood pressure between the 2 treatment arms could have resulted from:
  - The reduction of heart rate and the longer ventricular ejection time produced by the ß-blocking agent may have delayed the peak of the forward wave, thus inducing an AI increase and contributing to the AI differences between atenolol and Per/Ind.
  - The contrasting changes in vascular structure and/or function between Per/Ind and atenolol might be an explanation for differential patterns of wave reflections.

Hypertension 2001;38:922–6.

REASON: Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind Study



*Change from baseline to end value for left ventricular mass (LVM) for patients with **left ventricular hypertrophy** (n = 124).*

The tests were adjusted for brachial systolic BP and heart rate variation without altering the difference (−8.7; P <0.05), which can reflect the effect on LVM beyond the peripheral hemodynamic changes.

Am J Hypertens 2004;17:660-7.

Advantages of Per/Ind in patients with type 2 diabetes

3

Improved coronary microcirculation

Advantages of Per/Ind in patients with type 2 diabetes

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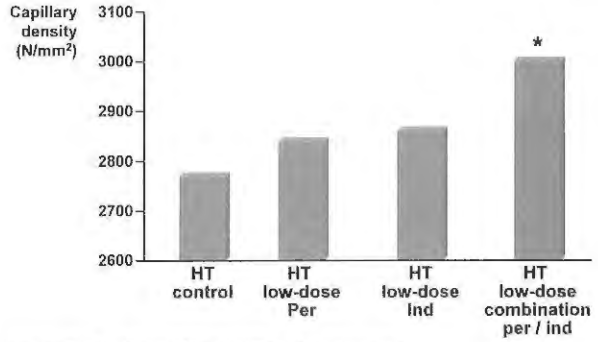
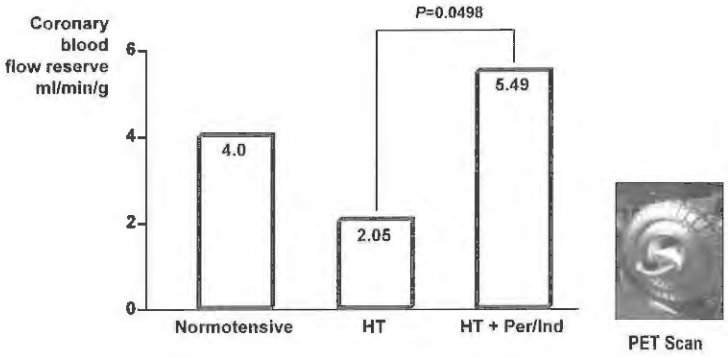
Effect of Per/Ind on capillary density in the myocardium in Stroke prone-SHR



*$P<0.05$ Bonferroni/Dunnet test *vs* control

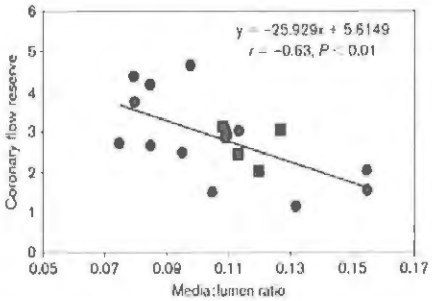
Microvasc Res 2000;59:243-54.

Effect of Per/Ind on myocardial perfusion in patients after 6 months of treatment



JRAAS 2003;4:9-95.

Media:lumen ratio & coronary flow reserve in patients with essential hypertension



- The alterations of vascular structure in peripheral microvessels may therefore be representative of parallel alterations in the coronary microcirculation.

• Coronary flow reserve = mean diastolic flow velocity after adenosine / resting mean diastolic flow velocity.
• A biopsy of subcutaneous fat from the gluteal region

J Hypertens 2003;21(3):625-31.

Advantages of Per/Ind in patients with type 2 diabetes

Renal protection

PREMIER: Preterax in albuminuria regression - Rationale -

Increased urinary albumin excretion is a major prognostic factor for:

- Progressive diabetic renal disease.
- Increased cardiovascular morbidity and mortality rates in both type 1 and type 2 diabetes.

Hypertension 2003;41:1063-71.

PREMIER: Preterax in albuminuria regression - TRIAL DESIGN -

Design
12-month international, randomized, double-blind, parallel group study.

Patients
481 Patients with type 2 diabetes with hypertension (140 mm Hg≤SBP<180 mm Hg and DBP<110 mm Hg) and albuminuria.

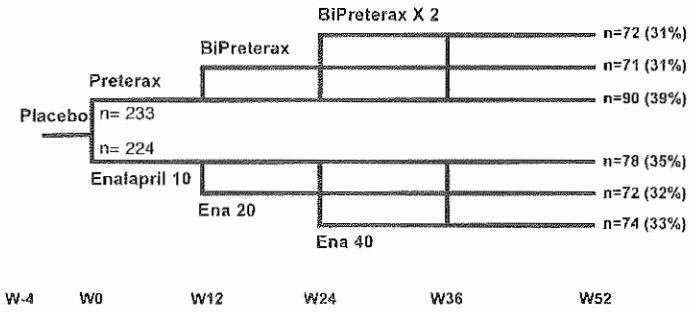
Follow up and primary endpoint
The change in the AER (g/min) after 1 year.

Treatment
Patients were randomly assigned in a double-blind manner to once-a-day therapy with either perindopril/indapamide (2/0.625 mg) or monotherapy with enalapril (10 mg).

Hypertension 2003;41:1063-71.

Advantages of Per/Ind in patients with type 2 diabetes 27 มีนาคม 2551

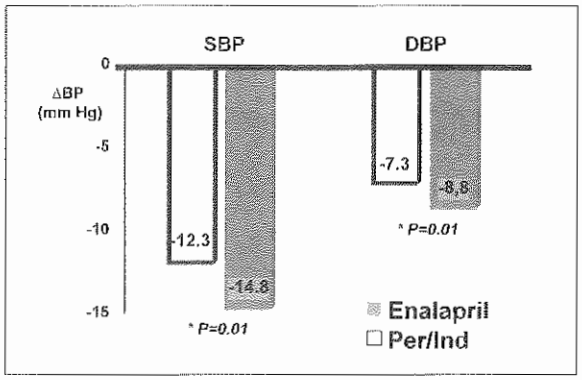
PREMIER: Preterax in albuminuria regression - TRIAL DESIGN -



Dose adjustment was permitted in patients whose SBP remained ≥140 mm Hg and/or DBP ≥90 mm Hg.

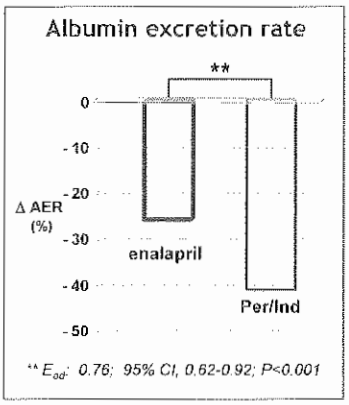
Hypertension 2003;41:1063-71.

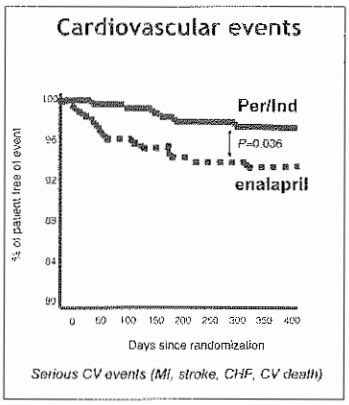
PREMIER: Preterax in albuminuria regression - RESULTS -



Hypertension 2003;41:1063-71.

PREMIER: Preterax in albuminuria regression - RESULTS -





Hypertension 2003;41:1063-71.

Advantages of Per/Ind in patients with type 2 diabetes

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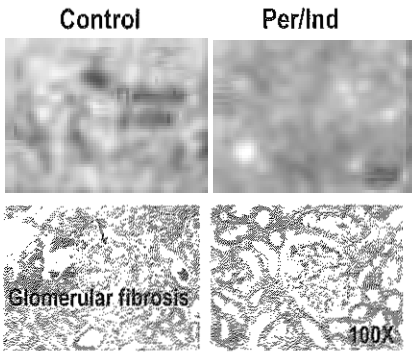
Protection of obese Zucker rat kidneys from fibrosis and renal failure with Per/Ind



An Per/Ind treatment was associated with complete kidney protection.

Fundam Clin Pharmacol 2004;18:437-47.

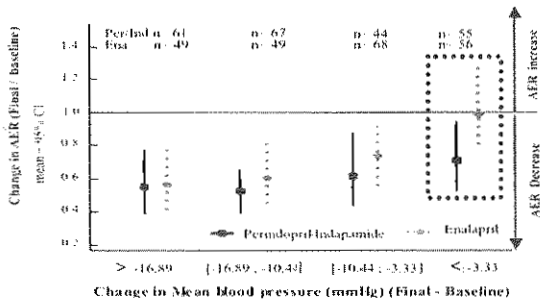
Protection of obese Zucker rat kidneys from fibrosis and renal failure with Per/Ind

Renoprotective effects associated with Per/Ind treatment can be attributed to renal structural protection:

- Prevents focal and segmental glomerular hyalinosis and tubulo-interstitial damage.
- Reduces several staining markers of glomerular and interstitial fibrosis.
- Reduces hypertrophy of superficial glomeruli and the mesangial expansion of deep glomeruli.
- Maintains glomerular filtration and renal hemodynamic parameters.
- Prevents occurrence of severe proteinuria.

Fundam Clin Pharmacol 2004;18:437-47.

PREMIER: Preterax in albuminuria regression - RESULTS -



An 30% reduction in AER even when the slightly fall in MAP (<3.3 mm Hg) raises the suggestion of a renoprotective mechanism independent of systemic BP lowering with Per/Ind.

Hypertension 2003;41:1063-71.

Advantages of Per/Ind in patients with type 2 diabetes

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ADVANCE

	Number of events: Per-Ind (n=5,569)	Placebo (n=5,571)	Favours Per-Ind	Favours Placebo	Relative risk reduction (95% CI)
Total renal events	**1243**	**1500**			**21% (15 to 27)***
New or worsening nephropathy	181	216			18% (-1 to 32)
New microalbuminuria	1094	1317			21% (14 to 27)

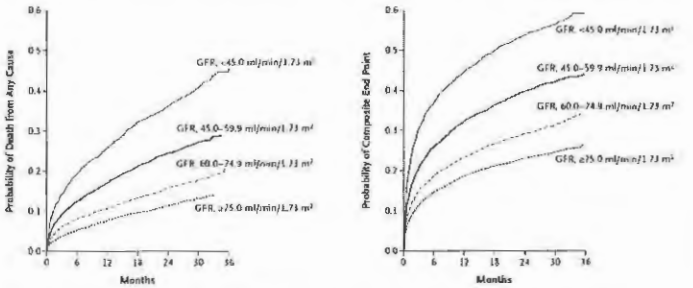
0.5 1.0 2.0

Hazard ratio

*p=<0.01

Lancet 2007;370:829-40.

Relation between Renal Dysfunction and Cardiovascular Outcomes after MI



- Renal disease should be considered a major risk factor for cardiovascular complications after MI.

N Engl J Med 2004;351:1285-95.

EUROPA: EURopean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease - TRIAL DESIGN -

Design

Multicenter, multinational, randomized, double-blind, placebo-controlled

Patients

12,218 patients with **previous MI**, angiographic evidence of **coronary artery disease**, **coronary revascularization** or a **positive stress test**; patients with heart failure excluded

Follow up and primary endpoint

Primary combined endpoint: cardiovascular death, MI or cardiac arrest. Mean 4.2 years follow up

Treatment

Placebo or **perindopril** 8 mg daily

Lancet 2003;362:782-8.

Advantages of Per/Ind in patients with type 2 diabetes

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Perindopril reduces risk of cardiovascular events in CAD patents irrespective of renal function

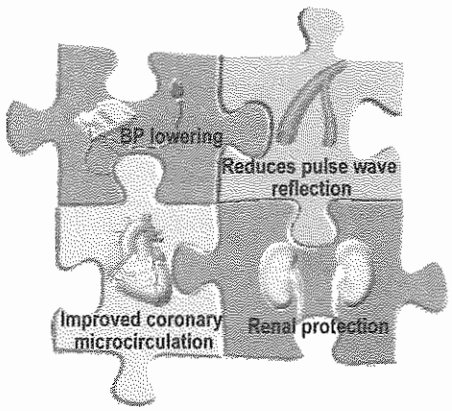
EUROPA

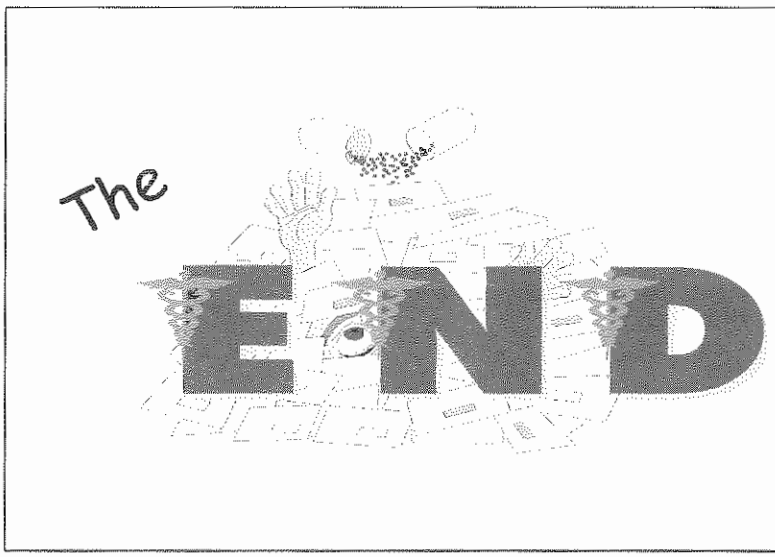
	Perindopril better / placebo better	Hazard ratio (95% CI)	Testing interaction
Primary end point			
≥75 ml/min/1.73m²		0.77 (0.64-0.93)	0.47 NS
<75 ml/min/1.73m²		0.84 (0.72-0.98)	
CV death, MI, stroke			
≥75 ml/min/1.73m²		0.79 (0.66-0.94)	0.48 NS
<75 ml/min/1.73m²		0.86 (0.74-0.99)	
Fatal and nonfatal MI			
≥75 ml/min/1.73m²		0.79 (0.63-0.98)	0.74 NS
<75 ml/min/1.73m²		0.75 (0.62-0.92)	

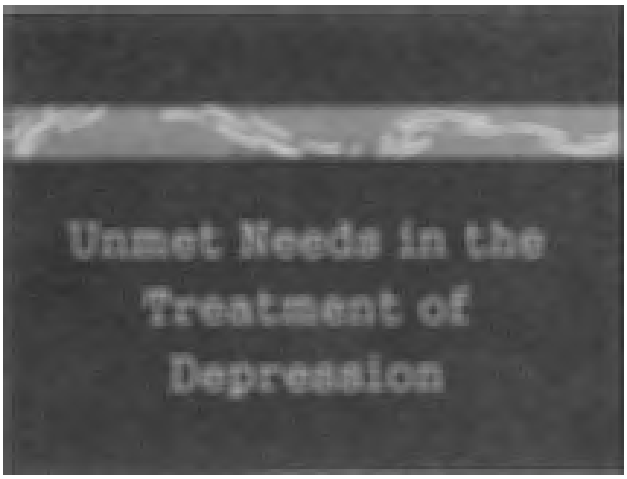
0.50 1.00 1.5

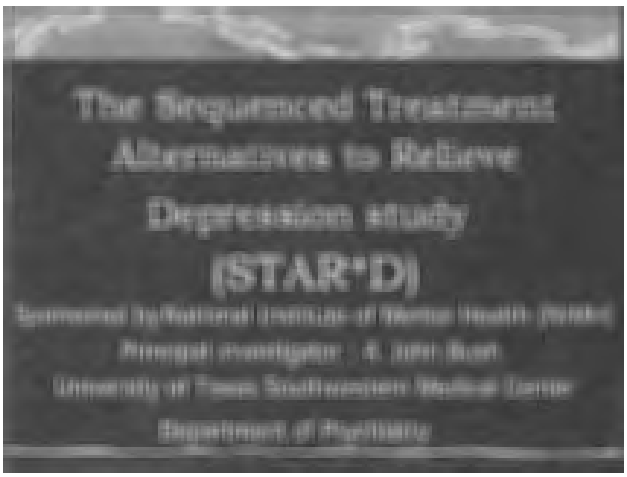
J Am Coll Cardiol 2007;50:2148-55.

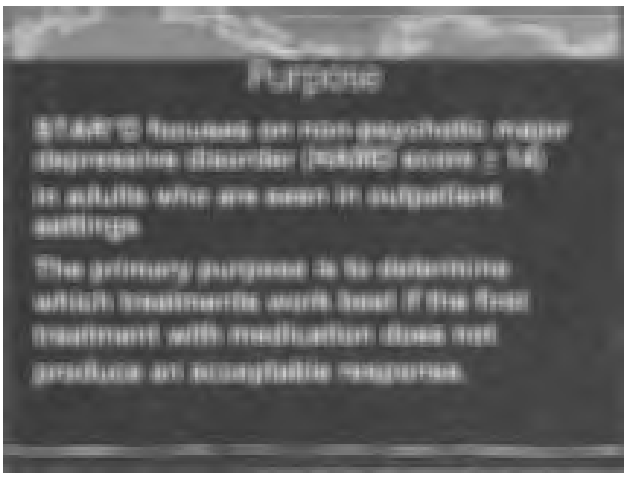
Advantages of Per/Ind in patients with type 2 diabetes

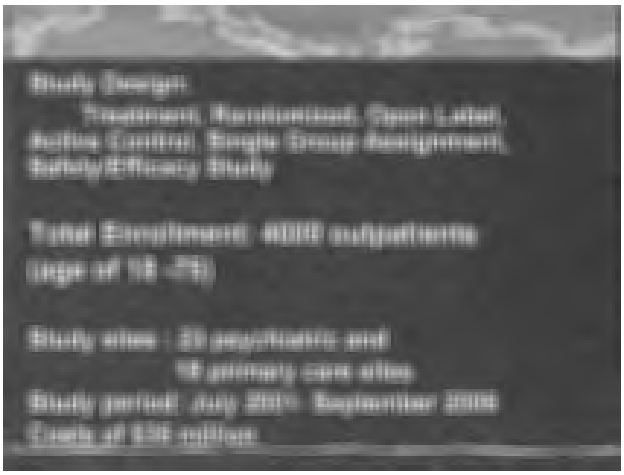


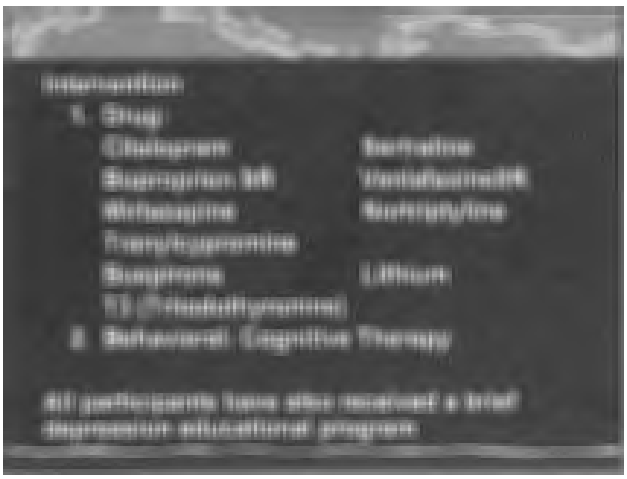


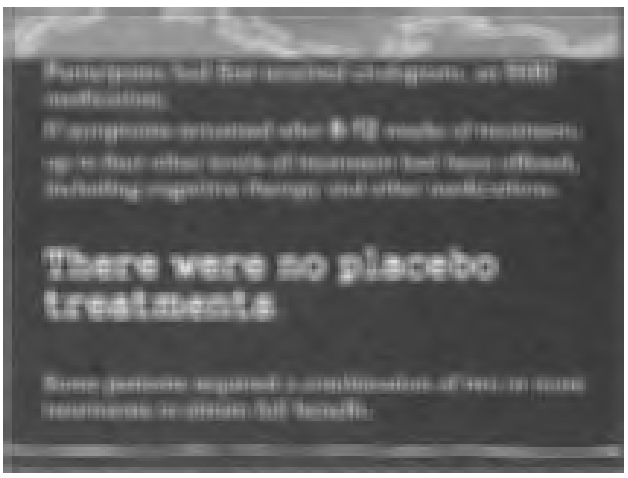

Unmet Needs in the Treatment of Depression

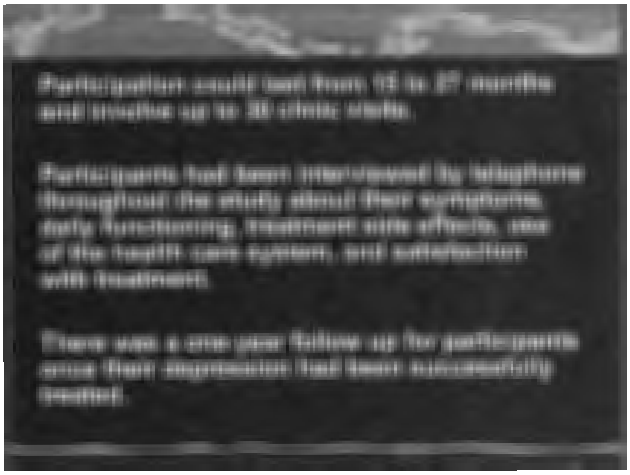

The Sequenced Treatment Alternatives to Relieve Depression study
(STAR*D)

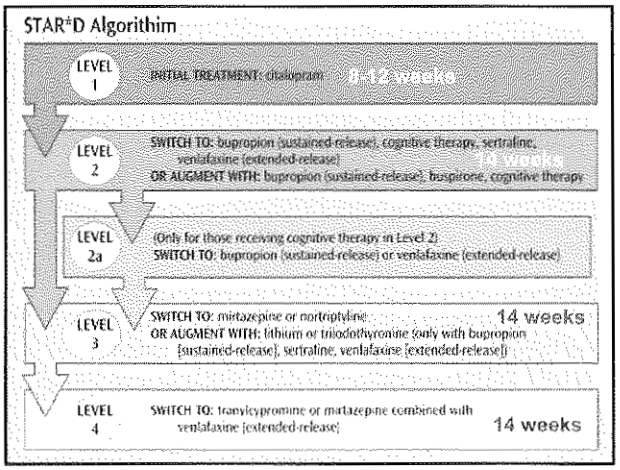

Purpose

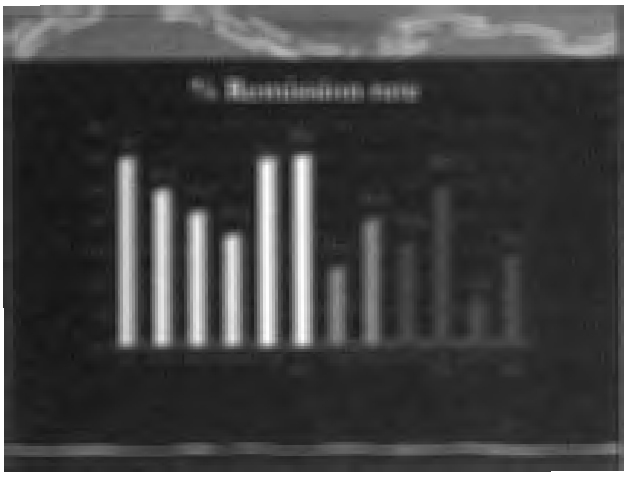


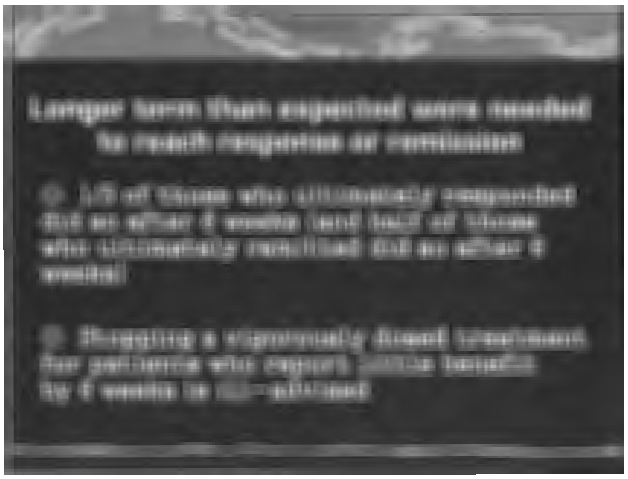


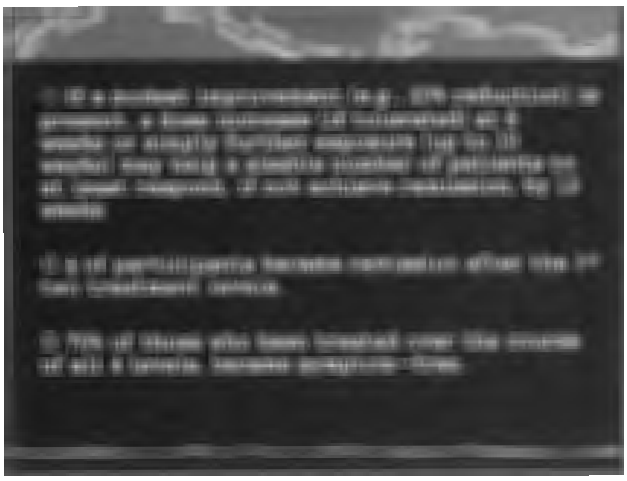

There were no placebo treatments




STAR*D Algorithm
LEVEL 1
INITIAL TREATMENT: citalopram
LEVEL 2
SWITCH TO: bupropion (sustained-release), cognitive therapy, sertraline, venlafaxine (extended-release)
OR AUGMENT WITH: bupropion (sustained-release), buspirone, cognitive therapy
LEVEL 2a
(Only for those receiving cognitive therapy in Level 2)
SWITCH TO: bupropion (sustained-release) or venlafaxine (extended-release)
LEVEL 3
SWITCH TO: mirtazepine or nortriptyline
OR AUGMENT WITH: lithium or triiodothyronine (only with bupropion [sustained-release], sertraline, venlafaxine [extended-release])
14 weeks
LEVEL 4
SWITCH TO: tranylcypromine or mirtazepine combined with venlafaxine (extended-release)
14 weeks







20–30% of patients remains unresponsive.
New strategies help treatment-resistant depressed patients become symptom-free
Switching to a different antidepressants

or

Additional medication
- Augmentation with ATD or non-ATD
- Combination 2 ATDs

Either a within-class switch (e.g., citalopram to sertraline) or an out-of-class switch (e.g., citalopram to bupropion-SR) is effective, as was a switch to a dual-action agent

A change in presumed mechanism of action at the third medication switch step (mirtazapine versus a third reuptake blocker, nortriptyline) did not produce different outcomes.

Thus, substantial pharmacologic differences between medications did not translate into substantial clinical differences in efficacy

"Better but not remitted" leads to a worse prognosis than full remission

Needs & Obstacles

Depression is slow to respond, even when optimal doses of conventional antidepressants are used, with 6 to 8 weeks is needed to establish a treatment respond

The time needed for up-titration to a therapeutic level

20–30% of patients remains unresponsive

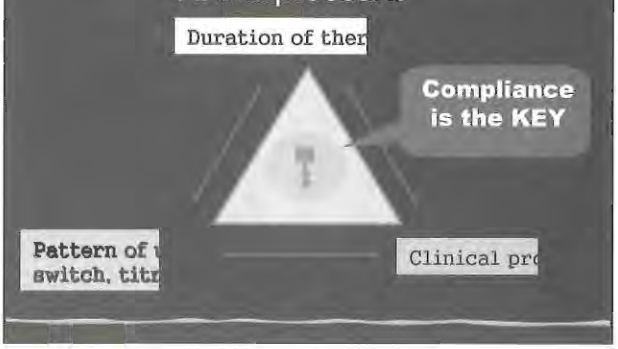
Needs & Obstacles

Side effects are unavoidable

Wide variability in pharmacokinetic and pharmacodynamic between patients

Which drug works best for whom can not be predicted in advance

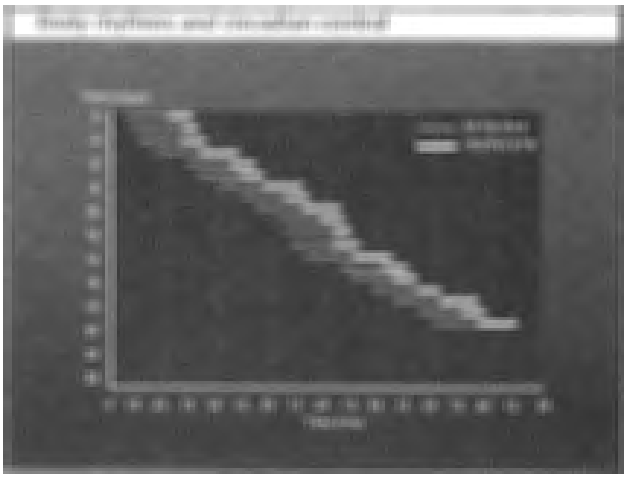
Effectiveness Component of Antidepressant

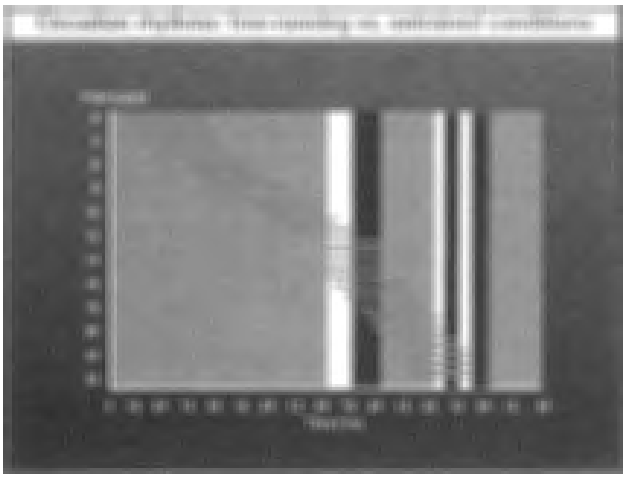


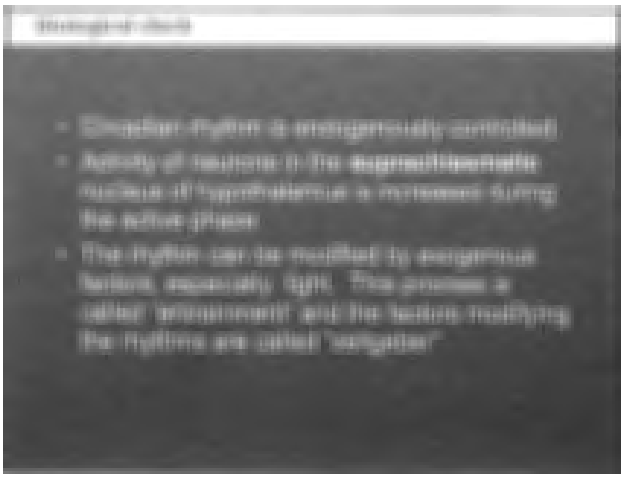

Depression & Circadian Rhythms


outline of talk


outline of talk







Defining the circadian rhythm

- Persistence in constant conditions with a period of about 24 hours.
- Ability to reset by exposure to a light or dark pulse
- Temperature compensated: proceeds at the same rate within a range of temperature

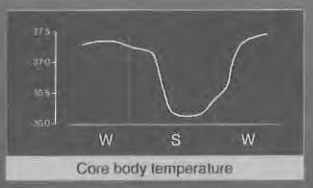
outline of talk:

Circadian rhythms
Suprachiasmatic nucleus
Depression and rhythm disturbance

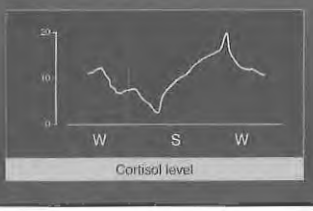
Suprachiasmatic nucleus

The suprachiasmatic nucleus of the hypothalamus (SCN) contains a master circadian pacemaker. Biological rhythms are synchronized (entrained) by light and darkness. This micrograph illustrates the way in which the SCN responds to the presentation of light during the night. The stained cells express the protein product of the immediate early gene, c-Fos, as an early step in shifting the biological clock

Circadian regulation of biological parameters

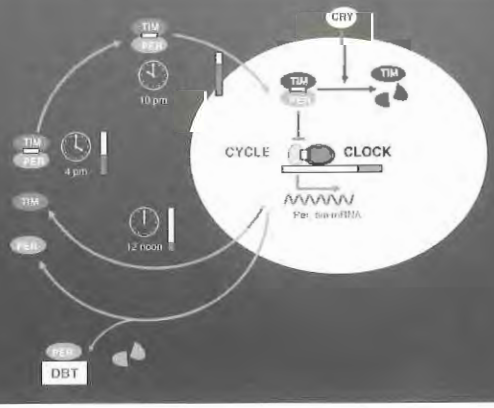


Core body temperature

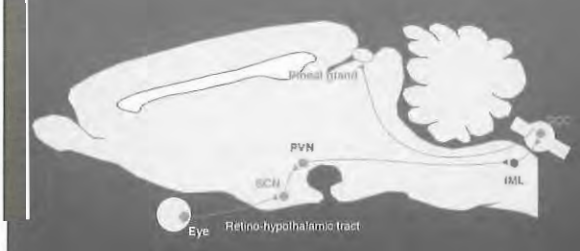


Cortisol level

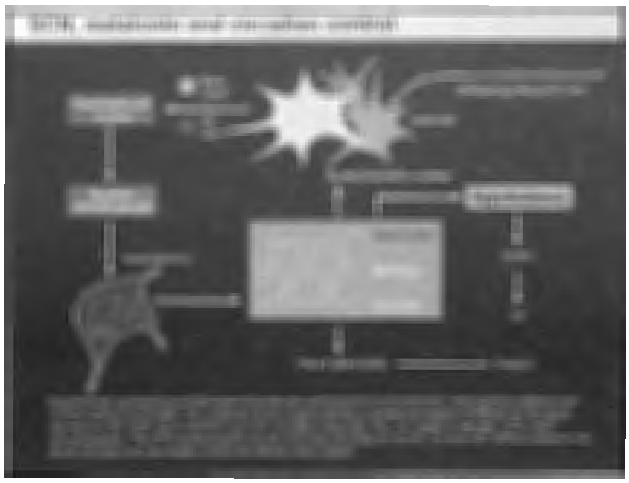
Proteins involved in circadian control

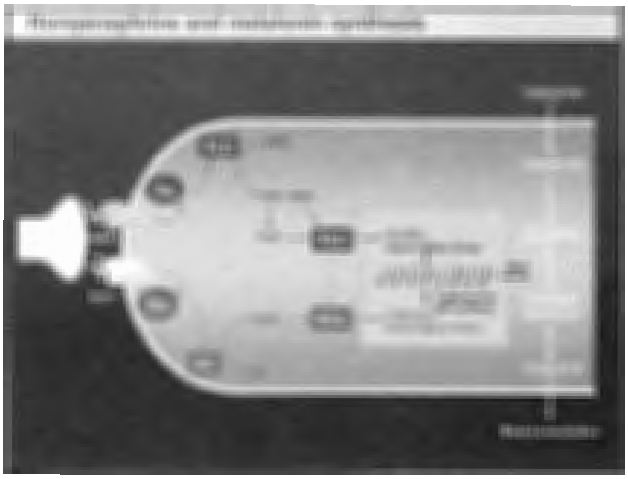


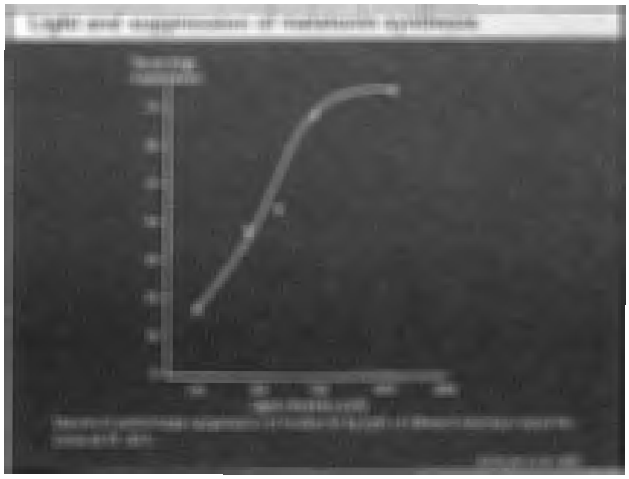
Light and pineal gland

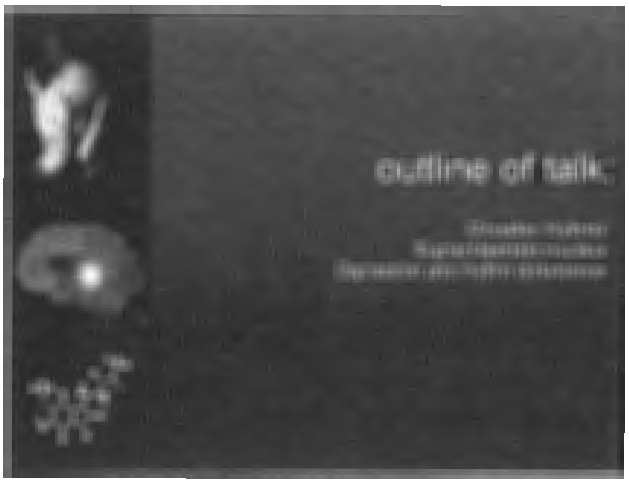


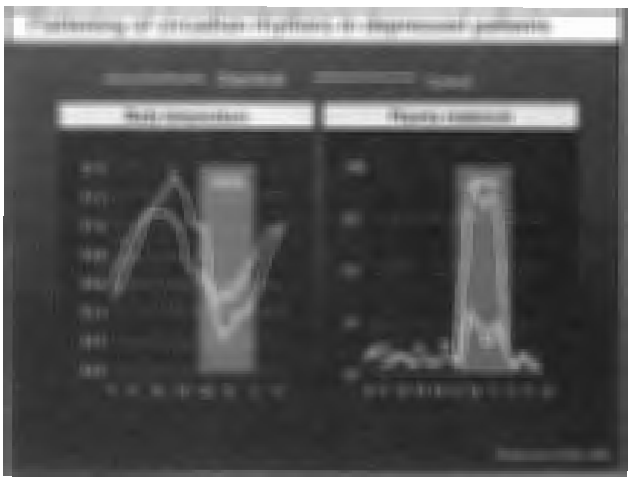
The neural system regulating pineal *N*-acetyltransferase in the rat. Light is detected by the eye, generating a signal that us transmitted by the retino-hypothalamic tract to the suprachiasmatic nucleus (SCN), which contains a circadian clock. The neural pathway includes the paraventricular nucleus (PVN). The intermediolateral cell column (IML), the superior cervical ganglia (SCC) and the pineal gland



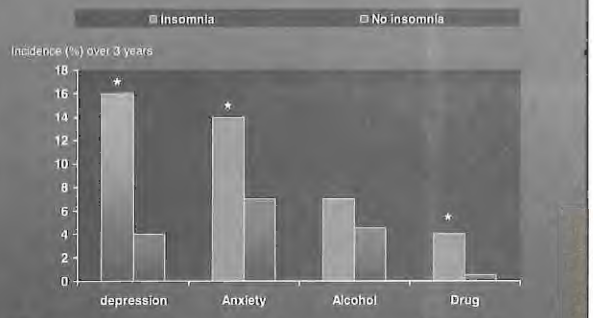





outline of talk:



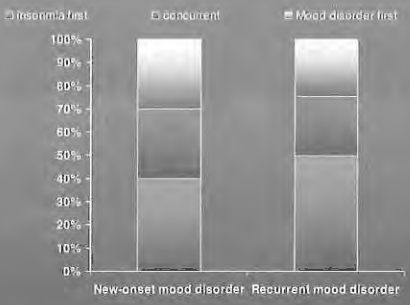
Insomnia as a risk factor of psychiatric disorders



The incidence of depression in patients with insomnia is around 16%, compared with only 4% in patients who do not suffer from insomnia. (Number of subjects: insomnia = 240, no insomnia =739)

Breslau, Bio Psychiatr 1996

Insomnia as a risk factor of psychiatric disorders



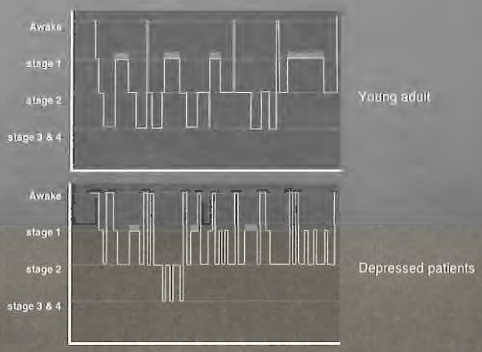
Mood disorder are often preceded by sleep disturbances. About 40% of patients with a new onset of mood disorders reported sleep disturbances before the onset of the mood disorder. Insomnia preceded mood disorders in 55% percent of patients with recurrent form.

Chayon and Roth, J Psychiatr Res 2003

Depressive episode – DSM IV criteria

- depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful). (In children and adolescents, this may be characterized as an irritable mood.)
- markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
- significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
- insomnia or hypersomnia nearly every day

Polysomnogram



Sleep-wake rhythm disturbance in depressed patients

- The sleep complaints in depressed patients
  - ↑ sleep latency
  - ↓ sleep maintenance
  - ↓ total sleep time
  - ↑ intra-sleep and early morning awakening
  - More common in older patients
  - Often very severe depression
- The daytime impairment in depressed patients
  - Difficult to distinguish from fatique
  - Subjective sleepiness often worse than shown by specific tests
  - More common on bipolar, young subjects

Wilson and Argyropoulos 2005

Clock genes and depression

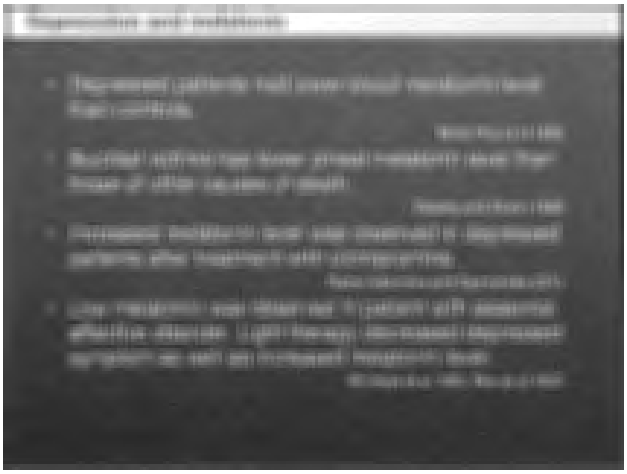
- Familial forms of circadian sleep disorders (i.e. advanced or delayed sleep phase syndrome) manifest allelic mutations on one or other clock genes.

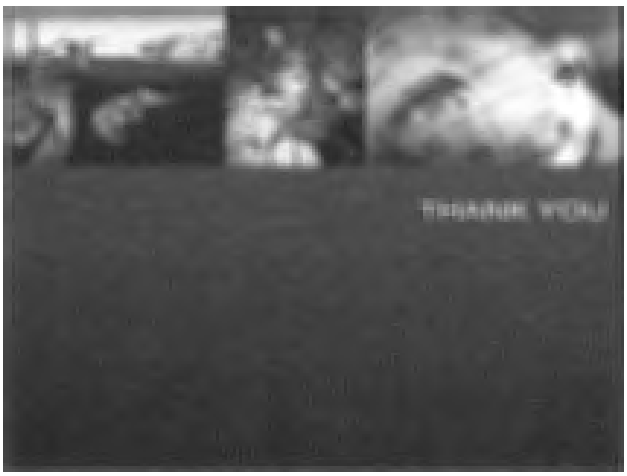
  Jones et al 1999, Ebisawa et al 2001
- No correlation between the *clock* gene mutation and major depression.

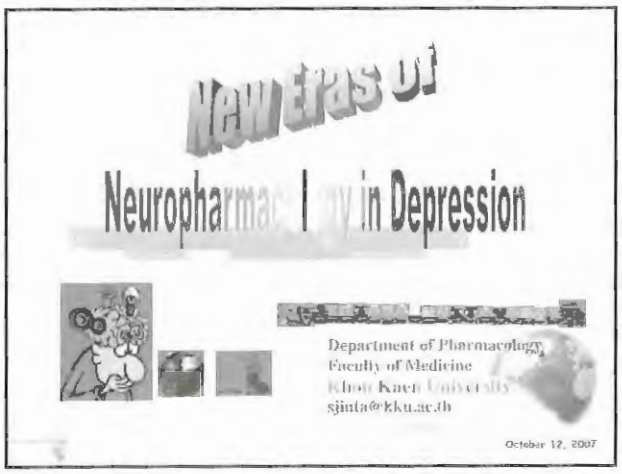
  Desan et al 2000, Baller et al 2005
- No correlation between the *per2* gene mutation and bipolar disorder.

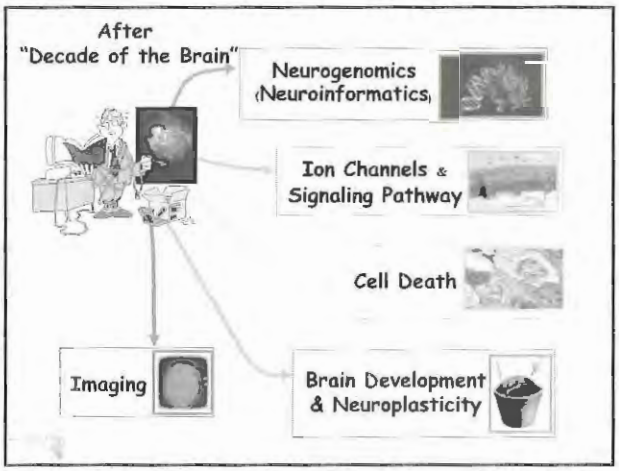
  Shiino et al 2003
- Circadian clock-related polymorphisms may related to susceptibility to seasonal affective disorders.

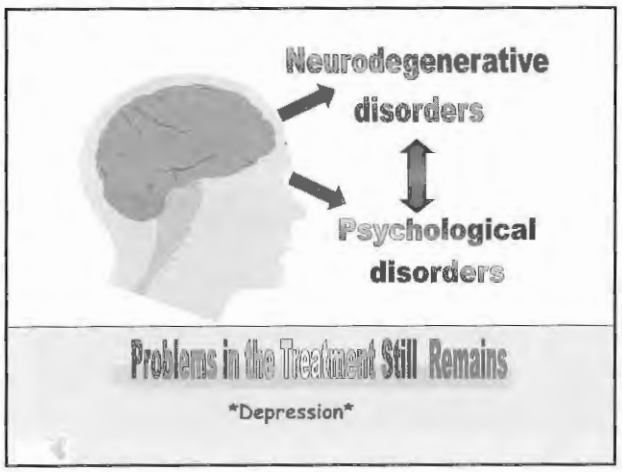
  Johansson et al 2003

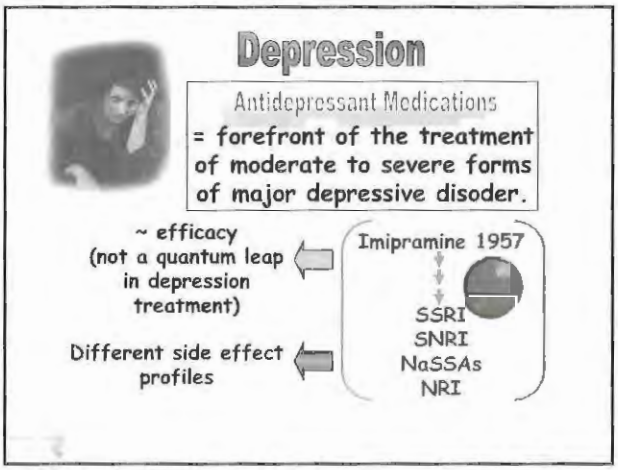




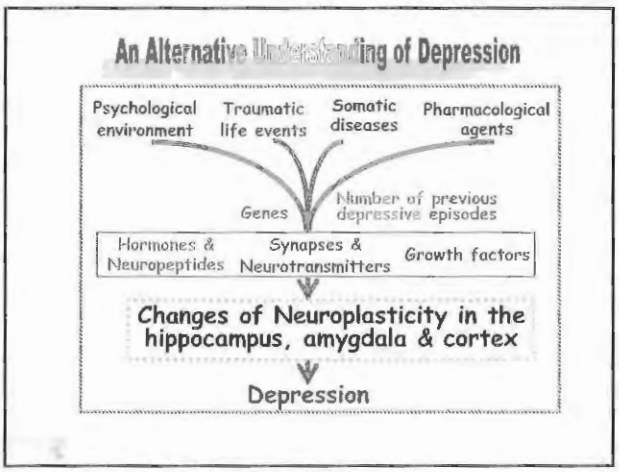

New Eras of
Neuropharmacology in Depression
Department of Pharmacology
Faculty of Medicine
Khon Kaen University
sjinta@kku.ac.th
October 12, 2007


After
"Decade of the Brain"
Neurogenomics
(Neuroinformatics)
Ion Channels &
Signaling Pathway
Cell Death
Imaging
Brain Development
& Neuroplasticity

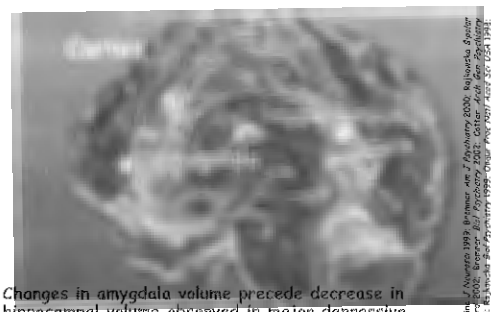

Neurodegenerative
disorders
Psychological
disorders
Problems in the Treatment Still Remains
Depression


Depression
Antidepressant Medications
= forefront of the treatment
of moderate to severe forms
of major depressive disoder.
~ efficacy
(not a quantum leap
in depression
treatment)
Different side effect
profiles
Imipramine 1957
SSRI
SNRI
NaSSAs
NRI


Current Status of Antidepressants
Significant shortcomings in Treatment Remains
Monoamine Hypothesis
Hindered the development of
novel drug treatments
Static
Response Rate
Slow onset
of Action
A Paradigm Shift


An Alternative Understanding of Depression
Psychological
environment
Traumatic
life events
Somatic
diseases
Pharmacological
agents
Genes
Number of previous
depressive episodes
Hormones &
Neuropeptides
Synapses &
Neurotransmitters
Growth factors
Changes of Neuroplasticity in the
hippocampus, amygdala & cortex
Depression

Depression is associated with alterations of neuroplasticity in brain areas involved in the control of mood and emotions ...



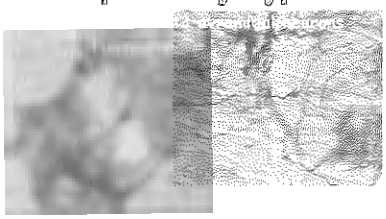
Changes in amygdala volume precede decrease in hippocampal volume observed in major depressive illness patients (McEwen, Biol Psychiatry 2003;54:200-207)

A New Generation of Antidepressant Drugs

- The Neuroplasticity Hypothesis
- Melatonin-based Hypothesis
- Neuropeptide Hypothesis

(Norman, 2006)

The Neuroplasticity Hypothesis



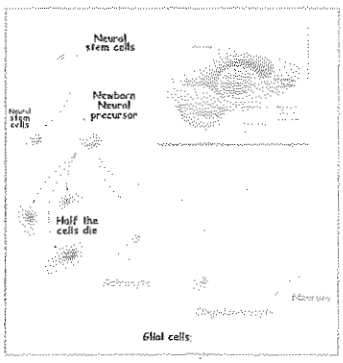
Brain Plasticity (Neuroplasticity)

- Neurogenesis (การสร้างเซลล์ประสาทใหม่)
- Synaptic plasticity (การปรับโครงสร้างของรอยประสานประสาท)

ความสำคัญของ Neuroplasticity (Used dependent property)

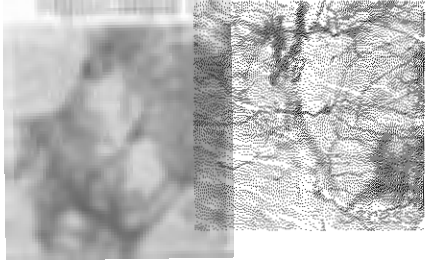
- -Brain development
- -Learning & memory (cognitive function)
- -Psychiatric disorders: Schizophrenia, Post-traumatic stress disorder & Depression
- -Neurological disorders: Parkinson's disease, Stroke, Alzheimer disease etc.
- -Cardiovascular disorders: Hypertension

Neurogenesis: stem cells in dentate gyrus



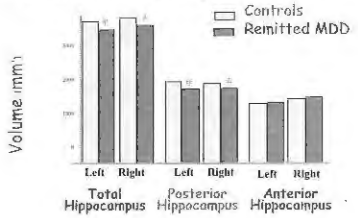
Dendritic remodeling

pyramidal neurons



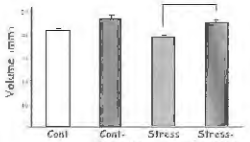
Reduced Hippocampal Volume in Unmedicated, Remitted Patients with Major Depression Versus Control Subjects

ใช้ high resolution MRI ใน unmedicated patients with remitted major depressive disorder



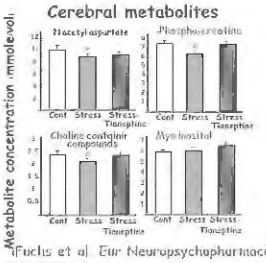
(Neumeister et al. Biol Psychiatry 2005)

Hippocampal volume



(Czeh et al. Proc Natl Acad Sci USA 2001)

Cerebral metabolites

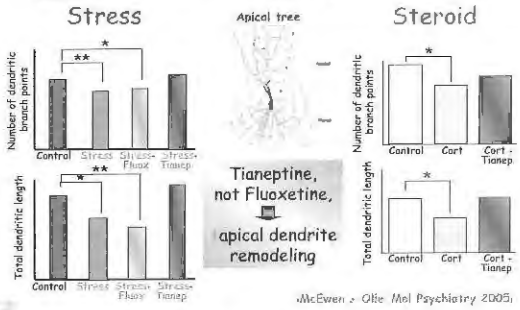


(Fuchs et al. Eur Neuropsychopharmacol 2004)

SSRE (tianeptine): ⇒ ↑ Cerebral metabolites, hippocampal volume & cell proliferation

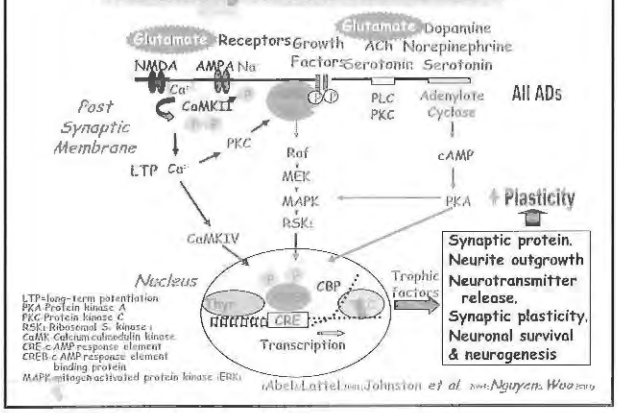
AD treatment ⇨ ↑ Neurogenesis

Effect of stress & ADs treatment on the number of dendritic branch point, length of CA_3 neurons & LTP.



(McEwen & Olie Mol Psychiatry 2005)

Plasticity & Neurotransmitters



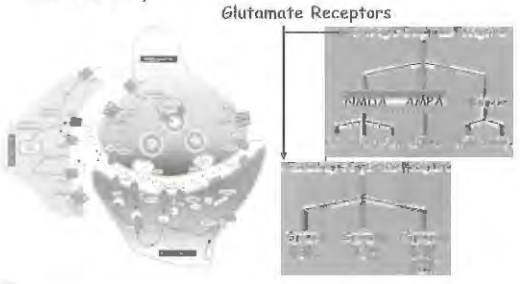
(Abel&Lattal; Johnston et al. ; Nguyen, Woo ...)

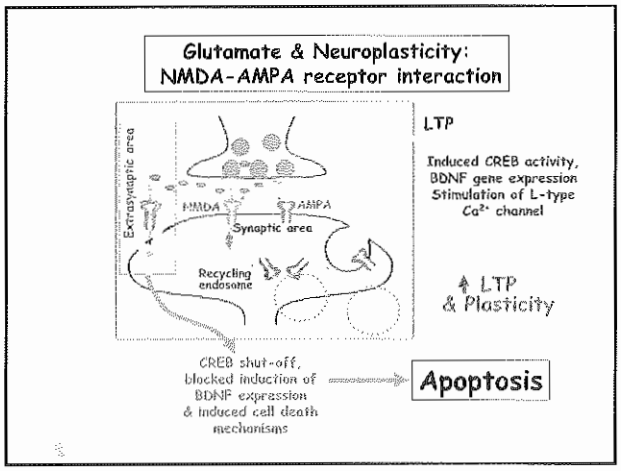
Glutamate systems

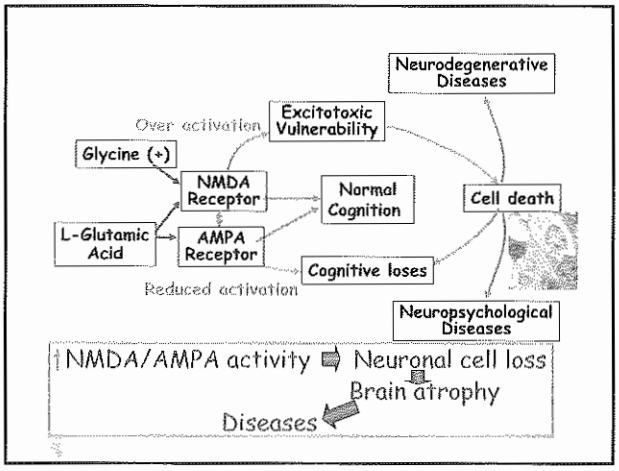
Glutamate (excitatory neurotransmitter) ⇒ LTP ⇒ ↑ Neuroplasticity

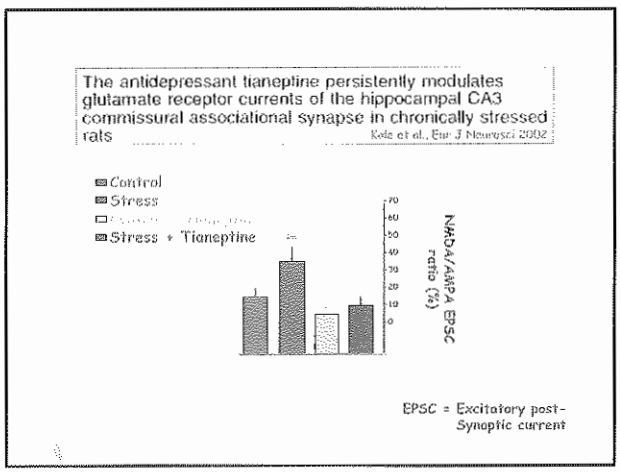
⇒ Neuronal cell death

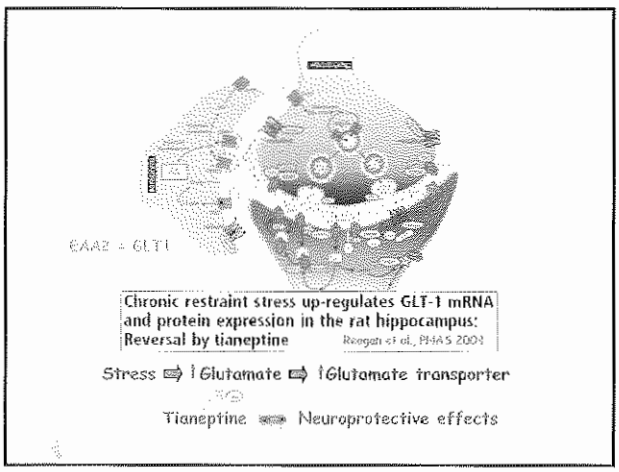
Glutamate Receptors

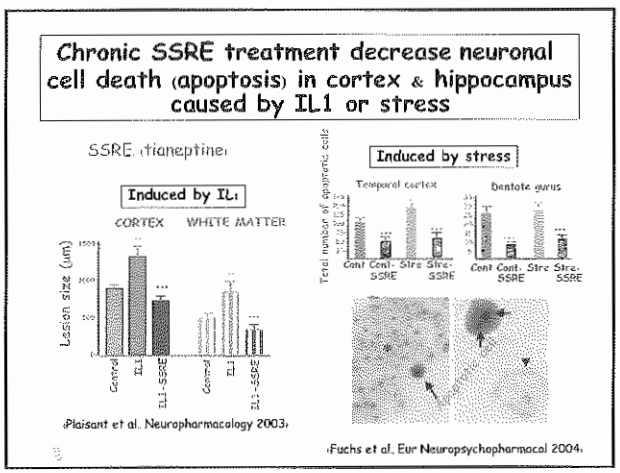


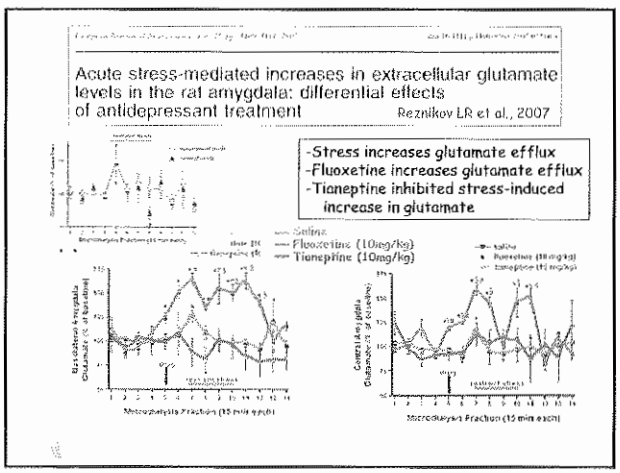

Glutamate & Neuroplasticity:
NMDA-AMPA receptor interaction
Extrasynaptic area
NMDA
AMPA
Synaptic area
Recycling endosome
LTP
Induced CREB activity, BDNF gene expression Stimulation of L-type Ca^{2+} channel
LTP & Plasticity
CREB shut-off, blocked induction of BDNF expression & induced cell death mechanisms
Apoptosis


Neurodegenerative Diseases
Over activation
Excitotoxic Vulnerability
Glycine (+)
NMDA Receptor
Normal Cognition
Cell death
L-Glutamic Acid
AMPA Receptor
Cognitive loses
Reduced activation
Neuropsychological Diseases
↑NMDA/AMPA activity ⇨ Neuronal cell loss
Brain atrophy
Diseases

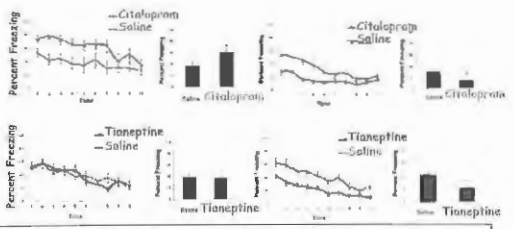

The antidepressant tianeptine persistently modulates glutamate receptor currents of the hippocampal CA3 commissural associational synapse in chronically stressed rats
Kole et al., Eur J Neurosci 2002
Control
Stress
Stress + Tianeptine
NMDA/AMPA EPSC ratio (%)
EPSC = Excitatory post-Synaptic current


EAAT2 = GLT1
Chronic restraint stress up-regulates GLT-1 mRNA and protein expression in the rat hippocampus: Reversal by tianeptine
Reagan et al., PNAS 2004
Stress ⇨ ↑Glutamate ⇨ ↑Glutamate transporter
Tianeptine ⇨ Neuroprotective effects

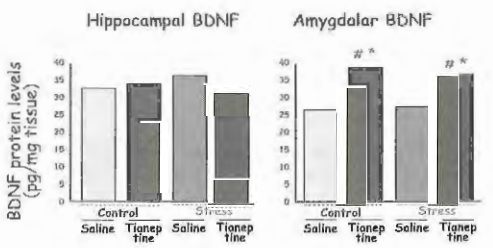

Chronic SSRE treatment decrease neuronal cell death (apoptosis) in cortex & hippocampus caused by IL1 or stress
SSRE (tianeptine)
Induced by IL1
CORTEX
WHITE MATTER
Lesion size (µm)
Control
IL1
IL1-SSRE
Induced by stress
Temporal cortex
Dentate gyrus
Cont
Cont-SSRE
Stre
Stre-SSRE
Plaisant et al. Neuropharmacology 2003
Fuchs et al. Eur Neuropsychopharmacol 2004


Acute stress-mediated increases in extracellular glutamate levels in the rat amygdala: differential effects of antidepressant treatment
Reznikov LR et al., 2007
-Stress increases glutamate efflux
-Fluoxetine increases glutamate efflux
-Tianeptine inhibited stress-induced increase in glutamate
Saline
Fluoxetine (10mg/kg)
Tianeptine (10mg/kg)

Amygdala Plasticity - Fear (anxiety)



SSRI: acute ⇨ ↓fear; chronic ⇨ ↓fear
SSRE: acute ⇨ no effect on fear; chronic ⇨ ↓fear



(Reagan LP et al., Eur J Pharmacol 2007;565:68-76)

The Neuroplasticity Hypothesis

Antidepressant treatment

Glutamate & BDNF

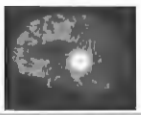
Increase in neurogenesis

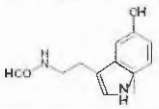
Increase in Dendritic remodeling

Reverse depressive disorders

Melatonin-based Hypothesis

(Monoamine-based Hypothesis ?)

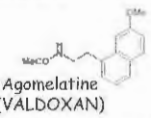




Melatonin, has positive effects on:
-Free radical cell damage
-Sleep problems caused by disturbances to circadian rhythms
-Seasonally determined changes to the central biological clock
-Stress etc.

Melatonin : no significant effect on depression

Agomelatine



Agomelatine (VALDOXAN)

Melatonin agonist (MT1 & MT2) easily cross the blood-brain-barrier

Resynchonizing altered rhythms in depression

5-HT2C antagonist

Anxiolytic

Enhance NA & DA release in frontal cortex

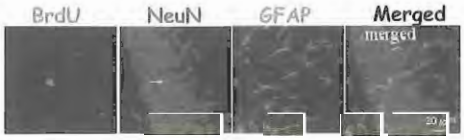
Antidepressant

Agomelatine targets a range of major depressive disorder symptoms

(Dubocovich, Curr Opin Investig Drugs, 2006)

Agomelatine & Neurogenesis

Chronic treatment with agomelatine & 3 weeks survival



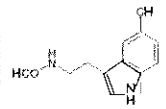
Confocal microscopy of bromodeoxyuridine (red) neuro specific nuclear protein (NeuN) (blue) glial fibrillary acidic protein (GFAP) (green) in hippocampal granule cell layer

Agomelatine ⇨ ↑Neurogenesis

Banasr et al.

The Novel Melatonin Agonist Agomelatine (S20098) Is an Antagonist at 5-Hydroxytryptamine$_{2C}$ Receptors, Blockade of Which Enhances the Activity of Frontocortical Dopaminergic and Adrenergic Pathways
Millan et al., J Pharmacol Exp Ther 2003

Depression and associated sleep disturbances: patient benefits with agomelatine
Kupfer Eur Neuropsychopharmacol 2006



Agomelatine, a New Antidepressant, Induces Regional Changes in Hippocampal Neurogenesis
Banasr et al., Biol Psychiatry 2006

Could agomelatine be the ideal antidepressant?
Pandi-Perumal et al., Expert Rev Neurother 2006

Neuropeptide Hypothesis

- Cholecystokinin (CCK) antagonists
- Substance P
- Neuropeptide Y

Many drugs are on pipeline, certain drugs are promising.

P01 Effects of 8(14), 15-isopimaradiene-3β, 18-diol on Functions of Mitochondria Isolated from Rat Liver

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[2]*Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*
E-mail: Withaya.J@Chula.ac.th

Abstract

Introduction: 8(14), 15-isopimaradiene-3β, 18-diol exhibited the antifeedant activity against termite and low phytotoxicity against lettuce seedings (1). However, there is no study of the pharmacological and toxicological mechanism on subcellular level such as mitochondria.

Objective: To investigate the effects of 8(14), 15-isopimaradiene-3β, 18-diol on functions of mitochondria isolated from rat liver.

Materials and methods: Mitochondria were isolated from rat liver. The respiration of mitochondria was measured polarographically at 37°C using a Clark-type oxygen electrode. Mitochondrial ATPase activity was measured the amount of inorganic phosphate (Pi) liberated from ATP hydrolysis (2).

Results: 8(14), 15-isopimaradiene-3β, 18-diol showed the inhibitory effect on both State 3 (ADP-stimulated) and State 3U [2,4-dinitrophenol (DNP)-stimulated] of mitochondrial respiration with glutamate combined malate as a substrate. In addition, this compound also inhibited the NADH oxidation of osmotic-shocked mitochondria. However, this inhibition could not be observed when the substrate was substrate. This compound had no effect on both mitochondrial ATPase activity and DNP stimulated mitochondrial ATPase activity. Respiratory Control Index (RCI) was decreased correlated with concentration of this compound.

Conclusion: 8(14), 15-isopimaradiene-3β, 18-diol inhibited oxidative phosphorylation and act as electron transport chain inhibitor at site I (Complex I).

Keywords: 8(14), 15-isopimaradiene-3β, 18-diol, *Xylia xylocarpa*, Mitochondria, Rat liver, Oxidative Phosphorylation.

Introduction

Mitochondria are essential organelles which play an important role in cell metabolism by mediated a number of metabolic functions (3). Mitochondria are the energy powerhouses of cells and make approximately 95% of the cell's ATP, utilizing oxidative phosphorylation (4). This process is carried out by the oxidative phosphorylation complexes. Free energy released by oxidation of substrates within the mitochondrial matrix is used to reduce the electron carriers, NAD (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) to form NADH and $FADH_2$ which, in turn, donate electrons to Complex I (NADH-ubiquinone oxidoreductase) and Complex II (succinate-ubiquinone oxidoreductase), respectively. Electrons from Complexs I and II are transferred to Complex III (ubiquinol-cytochrome *c* oxidoreductase) and Complex IV (cytochrome *c* oxidase), and the energy that is released during electron transfer is used to build a chemiosmotic gradient across the inner mitochondrial membrane. This gradient is used by Complex V (ATPsynthase/ F_1F_0-ATPase) for ATP synthesis (5). In addition to ATP synthesis, mitochondria are also critical to the modulation of cell osmotic regulation, redox status, pH control, cell signal transduction and

Ca^{2+} homeostasis. Disruption of mitochondrial bioenergetics has been recognized to participate in the lethal cell injury induced by xenobiotics, leading to cellular ATP depletion and cell death (6).

8(14), 15-isopimaradiene-3β, 18-diol, a diterpene substance extracted from heartwood of *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I.C.Nielsen., exhibited the antifeedant activity against termite and low phytotoxicity against lettuce seedings (1). However, there is no study of the pharmacological and toxicological mechanism on subcellular level. Accordingly, we designed this study to investigate the effects of 8(14), 15-isopimaradiene-3β, 18-diol on bioenergetic functions of mitochondria isolated from rat liver. It will contribute toward understanding of the pharmacological and/or toxicological mechanism on subcellular level (mitochondria).

Materials and Methods

Materials

adenosine 5' diphosphate (ADP), adenosine 5' triphosphate (ATP), bovine serum albumin (BSA), 2,4-dinitrophenol (DNP), HEPES, L-glutamic acid, magnesium chloride ($MgCl_2$), malic acid, nicotinamide adenine dinucleotide reduced form (NADH), oligomycin, potassium chloride (KCl), potassium phosphate monobasic anhydrous (KH_2PO_4), rotenone and succinic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 8(14), 15-isopimaradiene-3β, 18-diol was a gift from Assoc. Prof. Chaiyo Chaichantipyuth, Ph.D. All other chemicals in this study were of reagent grade and were locally and commercially available. 8(14), 15-isopimaradiene-3β, 18-diol, rotenone and oligomycin were prepared in absolute ethanol. The others were prepared in ultrapure water. Male albino Wistar rats weighing 200-250 g were used. They had free access to food and water in an animal room that was maintained at 25 ± 2°C with a 12-h light-dark cycle.

Preparation of rat liver mitochondria

Rat liver mitochondria were prepared from fresh livers essentially by the method of Hogeboom (7) as modified by Myers and Slater (8). The isolated mitochondria were suspended in 0.25 M sucrose at 20-30 mg protein/ml. Mitochondrial protein was determined by Lowry's method (9) as modified by Miller (10) using bovine serum albumin as a standard.

Measurement of mitochondrial respiratory activity

Oxygen consumption was measured polarographically at 37°C from the fresh mitochondrial fraction in 2.0 ml of incubation medium consisting of 92 mM KCl, 40 mM HEPES buffer and 2 mM $MgCl_2$ at pH 7.2, using a Clark-type oxygen electrode. Mitochondrial respiration was initiated by adding 310 μM ADP + 620 μM inorganic phosphate (Pi) with 5.2 mM glutamate + 5.2 mM malate or 5.2 mM succinate as the respiratory substrate. Oxygen consumption measured in the presence of added ADP was defined as State 3 respiration, while that measured in the absence of added ADP was defined as State 4 respiration. The RCI was calculated as ratio of State 3 respiration to State 4 respiration, and used as a marker of mitochondrial respiratory activity (11). Uncoupled respiration (State 3U) was induced by adding 50 μM DNP. Mitochondrial respiration was calculated as the nanoatoms of oxygen per minute per milligram of protein.

Measurement of osmotic-shocked mitochondrial respiratory activity

Oxygen consumption was measured polarographically at 37°C from the fresh mitochondrial fraction in 2.0 ml of incubation medium consisting of 29.5 mM KCl, 40 mM HEPES buffer and 2 mM $MgCl_2$ at pH 7.2, using a Clark-type electrode. Mitochondrial

respiration was initiated by adding 104 μM NADH as the respiratory substrate. Mitochondrial respiration was calculated as the nanoatoms of oxygen per minute per milligram of protein.

Measurement of mitochondrial ATPase activity

The ATPase activity can be determined by measuring the amount of Pi liberated from ATP hydrolysis (2). In this study, Pi was employed and quantitated by the calorimetric method of Fiske and Subbarow (12).

Statistical analysis

All data are expressed as the mean ± S.E.M. Data were analyzed by one-way analysis of variance, and Scheffe's test was applied to determine differences between the groups or by Student's *t*-test to determine differences between two groups. A level of $P<0.05$ was accepted as indicating statistical significance.

Results

Effect of 8(14), 15-isopimaradiene-3β, 18-diol on mitochondrial respiratory activity

8(14), 15-isopimaradiene-3β, 18-diol at dose ≥ 102.69 μM and at dose ≥ 68.46 μM could significantly decreased rate of State 3 and State 3U respiration respectively but no change in state 4 respiration with glutamate combined malate (NAD^+-linked substrate) as shown in the Figure 1. However, this result could not be observed when the substrate was succinate (FAD^+-linked substrate) [data not shown]. RCI was decreased correlated with concentration ≥ 68.46 μM of this compound (Table 1). The 50% inhibitory concentration (IC_{50}) of the compound on State 3 and State 3U respiration with glutamate combined malate as a substrate were 137.21 and 90.54 μM respectively.

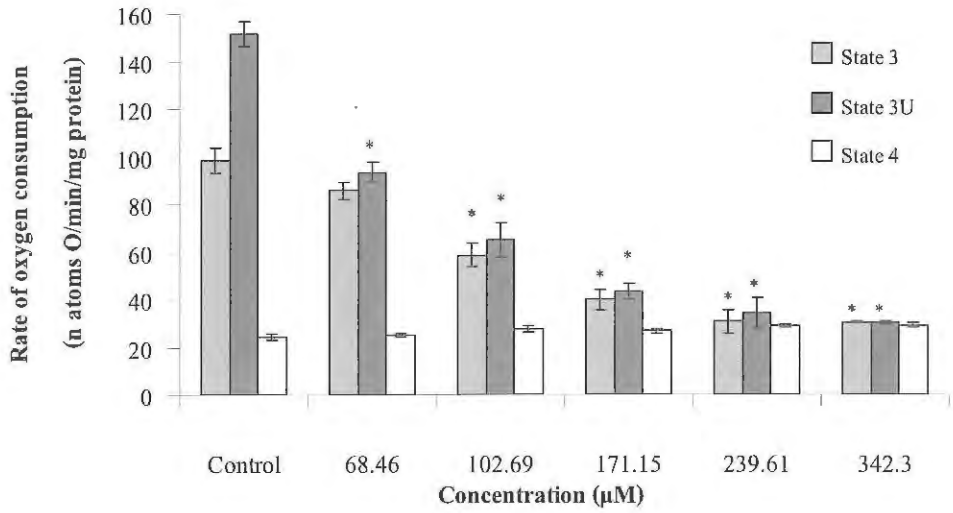

Fig. 1 Effect of 8(14), 15-isopimaradiene-3β, 18-diol on mitochondrial respiratory activity with glutamate combined malate as a substrate. Values are mean ± S.E.M. obtained from 5 rats. * $P<0.001$ compared with control.

Effect of 8(14), 15-isopimaradiene-3β, 18-diol on osmotic-shocked mitochondrial respiratory activity

8(14), 15-isopimaradiene-3β, 18-diol at dose ≥ 68.46 μM significantly decreased the respiratory stimulation evoked by NADH as shown in Table 2.

Table 1 The correlation between the concentration of 8(14), 15-isopimaradiene-3β, 18-diol and the RCI value with glutamate combined malate as a substrate

Concentration (μM)	RCI
0	5.78 ± 0.31
68.46	3.49 ± 0.11*
102.69	2.83 ± 0.14*
171.15	1.93 ± 0.10*
239.61	1.35 ± 0.07*
342.3	1.03 ± 0.06*

Values are mean ± S.E.M. obtained from 5 rats.
* $P<0.001$ compared with control.

Table 2 Effect of 8(14), 15-isopimaradiene-3β, 18-diol on osmotic-shocked mitochondrial respiratory activity with exogeneous NADH as a substrate

Concentration (μM)	Rate of oxygen consumption (n atoms O/min/mg proteins)
0	105.65 ± 11.88
68.46	31.31 ± 1.38*
102.69	22.65 ± 1.66*
171.15	19.85 ± 0.64*
239.61	19.39 ± 0.18*

Values are mean ± S.E.M. obtained from 5 rats.
* $P<0.001$ compared with control.

Effect of 8(14), 15-isopimaradiene-3β, 18-diol on mitochondrial ATPase activity

8(14), 15-isopimaradiene-3β, 18-diol at dose of 171.15 μM had no effect on both mitochondrial ATPase activity and DNP stimulated mitochondrial ATPase activity. In addition, DNP-stimulated ATPase activity was strongly inhibited by oligomycin significantly (Table3).

Table 3 The ATPase acticity of the various experiments

Experiments	ATPase activity (μ moles Pi /mg protein/10 min)
Control	4.20 + 0.45
0.17 mM DNP	13.17 + 0.83*
171.15 μM 8(14), 15-isopimaradiene-3β, 18-diol	5.33 + 0.17
0.17 mM DNP + 5 mg/ml oligomycin 2 μl	4.42 + 0.28**
0.17 mM DNP + 171.15 μM 8(14), 15-isopimaradiene-3β, 18-diol	14.48 + 1.92*

Values are mean ± S.E.M. obtained from 5 rats.
* $P<0.001$ compared with control , ** $P<0.001$ compared with 0.17 mM DNP

Disscussion

Oxidative phosphorylation of mitochondria can be inhibited by several inhibitors such as rotenone (inhibitor of Complex I), carboxin (inhibitor of Complex II), antimycin (inhibitor of Complex III), cyanide (inhibitor of Complex IV), oligomycin [inhibitor of Complex V (ATPsynthase/ F_1F_0-ATPase)] and uncouplers. Uncouplers such as DNP and dicumarol can separate oxidation from phosphorylation by acting as a proton-ionophore transporting protons across inner membrane to the matrix without passing through ATP synthase. Therefore ATP synthesis was inhibited and rate of electron transport was enhanced (13).

According to the results from this study, it was strongly indicated that 8(14), 15-isopimaradiene-3β, 18-diol had inhibitory effect on mitochondrial respiratory chain by inhibiting complex I of respiratory chain (Fig.1.) as same as rotenone while there was no effect on both ATPase activity and DNP stimulated ATPase activity (Table 3). It was

indicated that the compound had specific action on site I of respiratory chain while there was no effect on complex V (ATPsynthase/ F_1F_0-ATPase) which is a component of mitochondrial inner membrane. In addition, the compound did not stimulate state 4 respiration but DNP did. It was indicated that the compound is not uncoupler as DNP. According to the RCI value, it indicates that the tightness of the coupling mechanism, that is, whether the substrate oxidation is tightly coupled to ATP synthesis. The RCI value of good intact mitochondria should be at lease 4 at 37°C with glutamate combined malate as a substrate. From the results the RCI value was decreased correlated with concentration of the compound (Table 1). It was shown that the compound decreased tightness of the coupling mechanism of mitochondria.

It has been reported that the isolated compounds from the heartwood of *Xylia xylocarpa* including 8(14), 15-isopimaradiene-3β, 18-diol exhibited high antifeedant activity against termite and low phytotoxicity against lettuce seedings (1). It may be possible that a part of their toxicological mechanism is interfering with mitochondrial bioenergetics. In the propose study membrane potential changing should be study for investigate exactly pharmacological and/or toxicological mechanism. In addition, any derivatives from the heartwood of *Xylia xylocarpa* should also be explored.

Conclusion

8(14),15-isopimaradiene-3β, 18-diol inhibited oxidative phosphorylation and act as electron transport chain inhibitor at site I (Complex I).

Acknowledgements

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P02 Analgesic, Antipyretic, Anti-inflammatory and Toxic Effects of Andrographolide Derivatives in Experimental Animals.

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Abstract

Andrographolide, a major active component in *Andrographis paniculata* has been shown to possess many pharmacological effects including anti-inflammatory, antipyretic, analgesic and anti-HIV activities. The semisynthetic derivatives were synthesized to overcome the bitter taste. The aim of this study was to test for analgesic, antipyretic, anti-inflammatory, antidepressant, anxiolytic and toxic effects of these derivatives. In this study, andrographolide (SS1) and four semisynthetic derivatives (SS2, SS3, SS17 and SS19) were prepared in 5 % DMSO. Antipyretic and anti-inflammatory effects, in rat, were tested by using baker yeast-induced fever and carragenan-induced rat paw edema models, respectively. Analgesic, antidepressant and anxiolytic effects, in mice, were tested by using hot plate, forced swim and elevated plus maze models. Acute toxic effects were observed at up to 24 hours. The results showed that, at 4 mg/kg, all tested substances have significant analgesic effects and the highest potency was seen with SS3 and SS17. In baker yeast-induced fever model, only SS3 and SS17 could significantly reduced rats' rectal temperature ($p<0.05$). In carrageenan-induce inflammation model, SS1, SS3 and SS17 could significantly reduced paw volume. No antidepressant or anxiolytic effects could be seen with any substances tested. In acute toxicity test, SS3 and SS17, at a dose up to 100 mg/kg did not show any serious toxic effects. In conclusion, from this study, SS3 and SS17 are the most interesting 2 semisynthetic derivatives of SS1 which could be further developed to be analgesic, antipyretic and anti-inflammatory agents without any serious side effects.

Keywords: andrographolides, antipyretic, analgesic, anti-inflammatory, toxicity.

Introduction

Andrographis paniculata is a plant belongs to the family of *Acanthaceae,* that has been used as an official herbal medicine in China and Thailand for many years. Andrographolide, a diterpene lactones, is the major active component of this plant. It has been shown to possess many pharmacological effects including anti-inflammatory, antipyretic, analgesic and anti-HIV activities (1). It is recently reported to inhibit NF-kB binding to DNA thus reducing the expression of proinflammatory proteins such as cycloxygenase-2 (COX-2) (2). The problems limit the use of andrographolide was bitter taste. The semisynthetic derivatives were synthesized to overcome these problems.

The aim of this study was to test for analgesic, antipyretic, anti-inflammatory, antidepressant, anxiolytic and toxic effects of these derivatives.

Methods

In these experiments, andrographolide (SS1) and four semisynthetic derivatives (SS2, SS3, SS17 and SS19) used in this study were received from Faculty of Pharmaceutical Sciences, Khon Kaen University. The substances were suspended in 5 % DMSO and given to the animals by intraperitoneal injection. All animals were received only a single dose

treatment and each animal was used only once. Antipyretic and anti-inflammatory effects, in rat, were tested by using baker yeast-induced fever (3) and carragenan-induced rat paw edema models, respectively. Analgesic, antidepressant and anxiolytic effects, in mice, were tested by using hot plate, forced swim and elevated plus maze models. Acute toxic effects, if any, were observed after treatment until 24 hours. Data were tested by one-way ANOVA, Fisher LSD.

Results

At a dosage of 4 mg/kg all tested substances showed significant analgesic effects and SS3, SS17 showing the highest potency (Fig.1). In baker yeast-induced fever model, it appeared that, at doses 4mg/kg, only SS3 and SS17 but not SS1, SS2, SS19 could significantly reduced rats' rectal temperature when compared to 5% DMSO (control). The antipyretic effects of 4mg/kg SS3 and SS17 were comparable to the effect of 1.25 mmole/kg paracetamol (Fig. 2). It was shown that SS1, SS3 and SS17 (4mg/kg) could significantly reduced paw volume significantly when compared to 5% DMSO (Fig.3). No antidepressant or anxiolytic effects could be seen with any substances tested. In acute toxicity test, SS3 and SS17, at either 4, 8, 50, or 100 mg/kg, did not show any serious toxic effects.

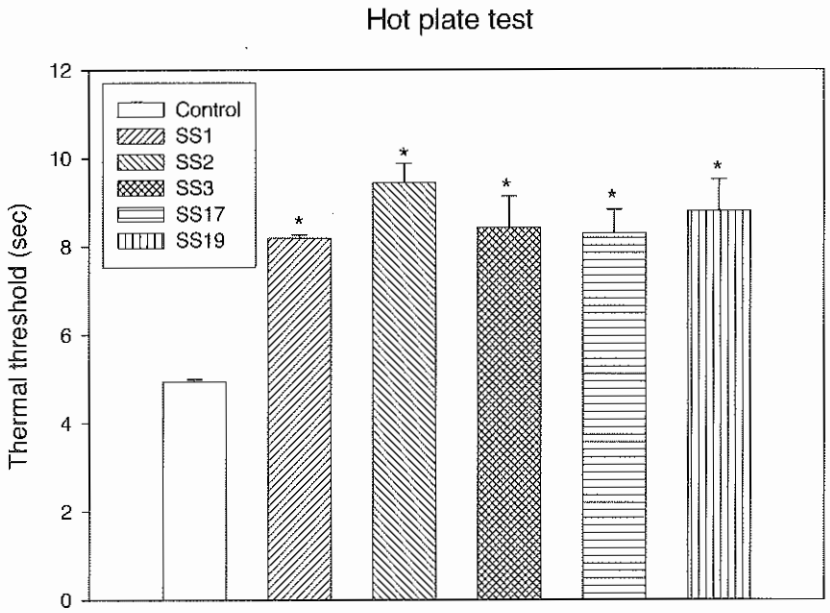

Figure 1 Thermal threshold of rats treated with either 5% DMSO, SS1, SS2, SS3, SS17 or SS19 in hot plate test. SS1, SS2, SS3, SS17 and SS19 could increase thermal threshold significantly ($p<0.05$) when compared to 5% DMSO (control).

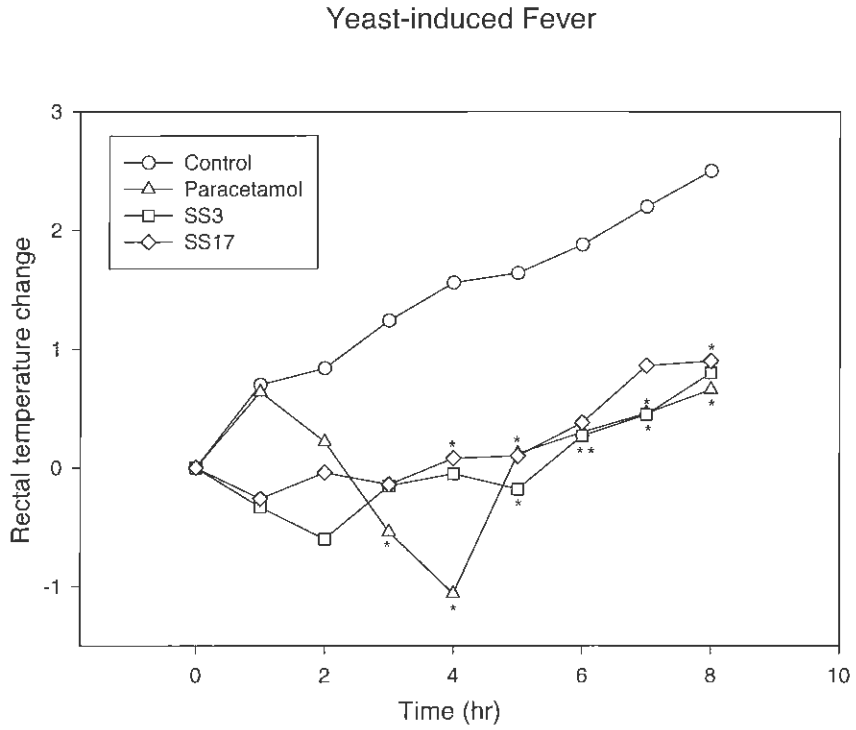

Figure 2 Rectal temperature changes of rats in baker yeast-induced fever model after treated with paracetamol, SS3 or SS17 could reduced rats' rectal temperature significantly ($p<0.05$) when compared to 5% DMSO (control).

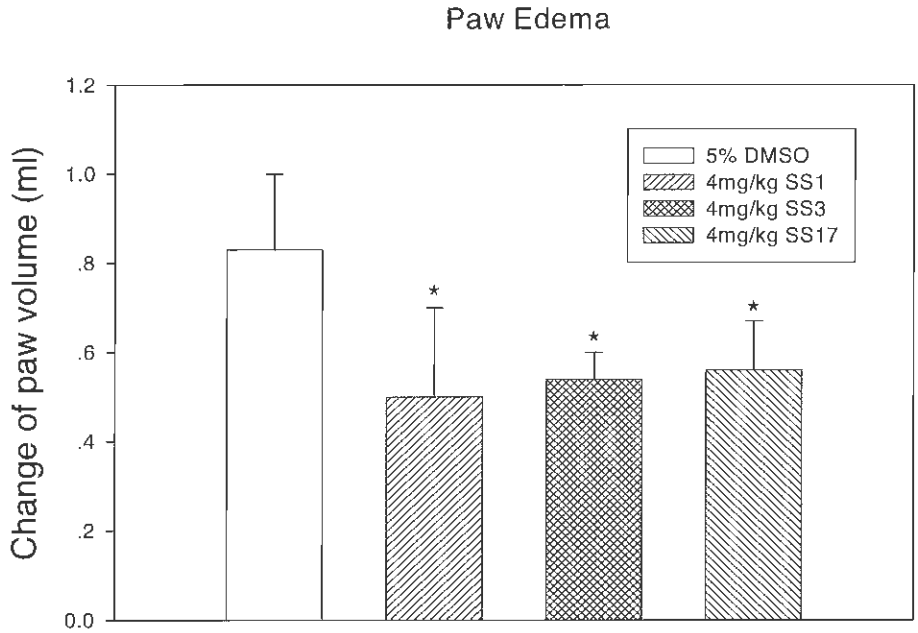

Figure 3 The changes of rat's paw volume treated with either SS1, SS3 or SS17 at 3 hrs after carageenan injection. SS1, SS3 and SS17 (4mg/kg) could reduced paw volume significantly ($p<0.05$) when compared to 5% DMSO (control).

Discussion

Modification of the structure to get non-bitter derivatives, SS2, SS3, SS17 and SS19 showed comparable pharmacological effects with andrographolide (SS1), although slightly differences. Interestingly, SS3 and SS17 appeared to be quite potent derivatives, even more than andrographolide itself. In addition, SS3 and SS 17 also showed antipyretic effect while andrographolide did not.

Conclusion

From this study, SS3 and SS17 are the most interesting 2 semisynthetic derivatives of andrographolide which could be further developed to be analgesic, antipyretic and anti-inflammatory agents without any serious side effects.

Acknowledgements

This project was partly supported by the Thailand Research Fund.

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P03 Antidiabetic Effect of Ethanolic Extract of Roselle (*Hibiscus sabdariffa*) in Chronic Streptozotocin-Induced Diabetic Rats

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Abstract

Introduction : Diabetes mellitus is one of the hallmarks of metabolic syndrome, which also include hyperlipidemia and hypertension. Roselle (*Hibicus sabdariffa*) extract has been reported to possess antidyslipidemia, antiatherosclerosis and antihypertensive activities. It is, therefore, interesting to investigate antidiabetic activity of roselle to support its use in the treatment of metabolic syndrome.

Objectives : To investigate antihyperglycemic and glucose tolerance improving activities of ethanolic extract of calyx of *H. sabdariffa* (HS-EE) in streptozotocin (STZ)-induced diabetic rats.

Methods : Male Sprague-Dawley rats (200-250 g) were induced to be diabetics by an i.p. injection of STZ 45 mg/kgBW. HS-EE was orally administered to diabetic rats for 6 weeks. Venous blood was collected to determine FBG and oral glucose tolerance test (OGTT). Insulin and metformin were used as reference hypoglycemic drugs.

Results : The administration of HS-EE at doses of 0.5 and 1.0 g/kgBW, but not 0.1 g/kg, for 6 weeks decreased the FBG of diabetic rats significantly ($p<0.05$) by 15±3 and 32±2 %, respectively, whereas insulin 4 U/kg caused a 30±7 % decrease of FBG in diabetic rats. For OGTT, 2 hour blood glucose levels of diabetic rats receiving HS-EE 0.5 and 1.0 g/kg were similar to their FBG. However, the 2 hour blood glucose of control diabetic rats (466±30 mg/dl) remained significantly higher than FBG level (334±40 mg/dl).

Conclusion : The ethanolic extract of *H. sabdariffa* possesses antidiabetic activity in chronic STZ-induced diabetic rats. Thus, the roselle extract may be useful in the management of metabolic syndrome.

Keywords: *Hibiscus sabdariffa*, Diabetes mellitus, Antidiabetic activity

Introduction

Metabolic syndrome is characterized by a clustering of a number of metabolic abnormalities (i.e. dyslipidemia, hyperglycemia) in the presence of underlying insulin resistance with a strong association with diabetes and cardiovascular disease. *Hibiscus sabdariffa* Linn (roselle), locally called "Krachiap Daeng" in Thailand, belongs to the Family Malvaceae. Interestingly, the extract of roselle calyx has been reported of antidyslipidemia (1), antiatherosclerosis (2) and antihypertensive (3) activities. Thus, the investigation of antidiabetic activity of roselle may add more support in applying roselle extract in the treatment of metabolic syndrome. Therefore, this study had an aim to investigate the antidiabetic effect of ethanolic extract of *H. sabdariffa* in streptozotocin (STZ)-induced diabetic rats.

Methods

1) Plant material and extraction : One year old *H. sabdariffa* (HS) was collected from Khoun Meed District, Songkla Province, in September 2007. The dry calyx of *H. sabdariffa* was minced and immersed in absolute ethanol for 3 days, and then filtered. The left fiber was repeatedly extracted by ethanol for 3 times. Alcohol was removed from the filtrate using the rotary vacuum evaporator. The remaining alcohol was further evaporated at a controlled temperature of 50 °C. By this procedure, the dry powder (HS-EE) was obtained and the yield was 21.5%

2) Induction of diabetic rats : Male Sprague-Dawley rats (200-250 g) were obtained from the National Laboratory Animal Centre, Mahidol University, Bangkok. They were maintained in air-conditioned room (25±1° C) with relative humidity of 44-55% under a 12 h light: 12 h dark cycle. They received standard rat chow (C.P. mice feed, Thailand) and tap water 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. Rats were induced to be diabetics by a single i.p. injection of STZ 45 mg/kg BW. After 7 days of STZ injection, fasting blood glucose (FBG) level was determined using glucometer (Accu-check Advantage II; Roche). The animals with FBG over 200 mg/ml were included in the experiment.

3) Experimental Protocol

3.1) Examination of the effect of HS-EE on FBG of diabetic rats

The diabetic rats were divided into 5 groups with four to five animals in each group and on the following administration protocols for 6 weeks: distilled water (p.o.), insulin (Monotard®) 4 U/kg BW (s.c.) and HS-EE 0.1, 0.5 and 1.0 g/kg BW (p.o.). The FBG was determined at 1 and 6 weeks after all those administrations.

3.2) Examination of the effect of HS-EE on glucose tolerance of diabetic rats

The diabetic rats were divided into 5 groups and on the administration protocols as follows for 7 days: distilled water (p.o.), metformin 0.5 g/kg bw. (p.o.), and HS-EE 0.1, 0.5 and 1.0 g/kg bw. (p.o.). On the seventh day, the oral glucose tolerance test (OGTT) was performed. Thirty minutes after receiving the last dose of all administrations, the animals were loaded orally with glucose 2 g/kg BW. The blood glucose concentrations before (0) and at 30, 60 and 120 min after glucose loading were determined.

4) Statistical analysis

Results were presented as mean±S.E.M. The effect of HS-EE on FBG was analyzed by paired t-test, while effect on OGTT was analyzed by analysis of variance (ANOVA) and significance was accepted at $p < 0.05$.

Results

Effects of HS-EE on FBG. As shown in Table 1 and Table 2, the administration of HS-EE at doses of 0.5 and 1.0 g/kg for short term (1 week) or long term (6 weeks) significantly decreased the FBG of diabetic rats as compared to the control diabetic rats receiving distilled water. The percent decrease in FBG in response to HS-EE was dose-dependent (Table 1 and Table 2). The antihyperglycemic activity of HS-EE 1.0 g/kg was comparable to that of insulin 4 U/kg (Table 2).

Effect of HS-EE on OGTT. The administration of HS-EE 0.5 and 1.0 g/kg for 1 week significantly improved glucose tolerance of diabetic rats (Figure 1) as the blood glucose levels at 2 hr after glucose loading (254±10 and 293±45 mg/dl, respectively) were comparable to the levels at fasting stage (280±30 and 231±22 mg/dl, respectively). For the control diabetic rats, the 2 hour blood glucose (466±30 mg/dl) was significantly ($p<0.05$) higher than

its fasting blood glucose level (334±40 mg/dl). Metformin, an insulin sensitizing agent, also showed OGT improving activity with the 2 hr blood glucose of 254±10 mg/dl as compared to fasting blood glucose of 281±29 mg/dl.

Table 1 The effect of one week administration of HS-EE on FBG of diabetic rats.

Groups	N	FBG (mg/dl)		
		Before treatment	After 1 week of treatment	% decrease in FBG
Distilled water	5	413 ± 22	405 ±17.00	2 ± 2
Insulin 4 U/kg	5	465 ± 58	291 ±35**	35 ± 8*
HS-EE 0.1 g/kg	5	304 ± 10	313 ±18	0
HS-EE 0.5 g/kg	5	292 ± 34	252 ±30**	13 ± 3*
HS-EE 1.0 g/kg	5	331 ± 14	245 ±12**	26± 3*

Values are mean ± S.E.M.,
HS-EE: ethanolic extract of *H. sabdariffa*, N: number of animals, FBG: fasting blood glucose
*: $p<0.05$ as compared to control diabetic rats receiving distilled water
**: $p<0.05$ as compared to before treatment

Table 2 The effect of six weeks administrations of HS-EE on FBG of diabetic rats

Groups	N	FBG (mg/dl)		
		Before treatment	After 6 weeks of treatment	% decrease in FBG
Distilled water	5	413 ± 22	421 ±17	0
Insulin 4 U/kg	5	465 ± 58	314 ±32**	30 ± 7*
HS-EE 0.1 g/kg	4	304 ± 10	373 ±77	0
HS-EE 0.5 g/kg	4	292 ± 34	241 ±26	15 ± 3
HS-EE 1.0 g/kg	5	331 ± 14	227 ±12**	32 ± 2*

Values are mean ± S.E.M.,
HS-EE: ethanolic extract of *H. sabdariffa*, N: number of animals, FBG: fasting blood glucose
*: $p<0.05$ as compared to control diabetic rats receiving distilled water
**: $p<0.05$ as compared to before treatment

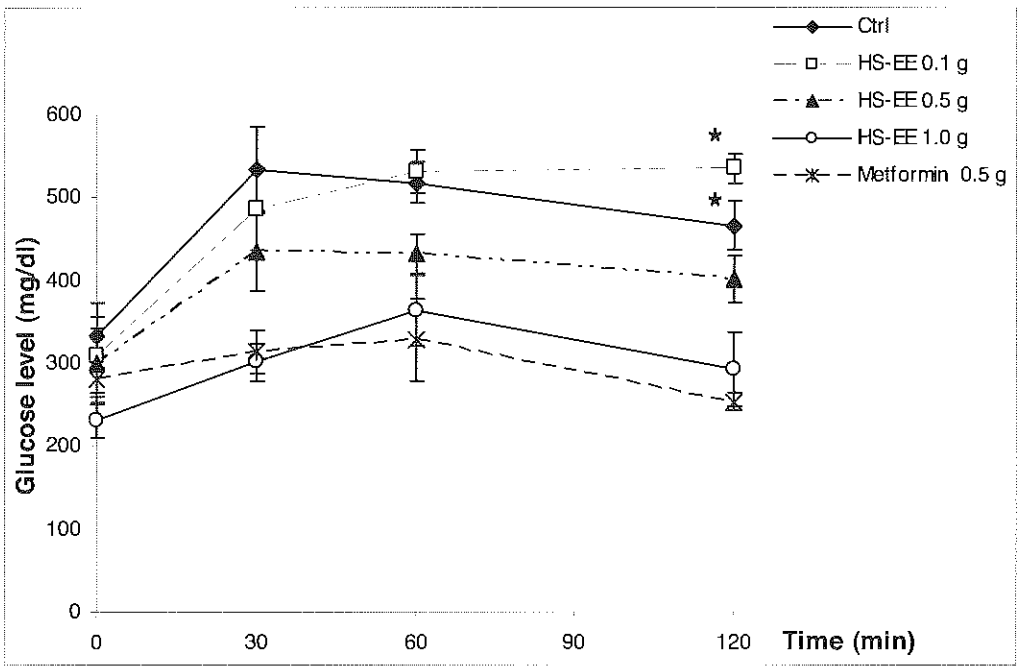

Figure 1 The effect of HS-EE on OGTT of diabetic rats. HS-EE (0.5 and 1.0 g/kg) and metformin (0.5 g/kg) significantly improved glucose tolerance of diabetic rats.

Values are mean ± S.E.M.
*: $p<0.05$ as compared to corresponding blood glucose at 0 min (fasting blood glucose)
Ctrl: control diabetic rats
HS-EE: ethanolic extract of *H. sabdariffa*
Number of animals in each group was five.

Discussion

The present study demonstrates the anti-hyperglycaemic and oral glucose tolerance improving activities of ethanolic extract of *H. sabdariffa* in streptozotocin-induced diabetic rats. STZ causes diabetes by damaging β-cells, which leads to the reduction of insulin release (4). Both short-term and long-term administration of HS-EE result in reduced plasma glucose in diabetic rats. The effective long-term regimen may especially be appropriate for treating diabetic patients who need chronic medication.

A glucose tolerance test in medical practice is the administration of glucose to determine how the body can dispose a loaded glucose from the blood (5). In non-diabetic person, the 2 hour glucose should not be higher than 140 mg/dl or should be similar to fasting blood glucose. In our experiment, control diabetic rats had higher 2 hr glucose level than its fasting blood glucose indicating defect in glucose disposal from blood. Interestingly, even short-term administration of HS-EE 1 g/kg could improve this glucose disposal within 2 hours. The mechanisms by which the extract uses to mediate the anti-hyperglycemia and glucose tolerance improvement are being investigated.

Conclusion

The ethanolic extract of *H. sabdariffa* calyx possesses antidiabetic activity in chronic STZ-induced diabetic rats. Thus, the roselle extract may be useful in the management of metabolic syndrome in the near future.

Acknowledgement

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P04 Neutralization Of Lethality Of *Naja Kaouthia* Venom By Dialdehyde Compound From *Curcuma Sp.* Rhizome.

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Abstract

Many plants of *Curcuma sp.* (Zingiberaceae) are reputed in Thailand as "snakebite antidote". The rhizomes of these plants were found to have a common yellow substance proofed to be a dialdehyde compound and beleieved to be an active substance. In this study, dialdehyde compound was tested both *in vitro* and *in vivo* for antivenom activity against *Naja kaouthia* venom. The compound (in 95% ethanol) has been found to have significant ($p < 0.01$) antagonistic effect on the inhibition of neuromuscular transmission produced by the venom in isolated rat phrenic nerve-hemidiaphragm preparations. Mice, subcutaneously injected with venom at 0.75 mg/kg, had the survival time of 52.00 ± 5.35 min, while giving dialdehyde compound at a dose of 100 mg/kg (in 5% DMSO) orally or intraperitoneally , 30 min before the venom, had survival time of 61.50 ± 5.38 and 88.75 ± 11.15 min, respectively. Mice received the mixture of venom and the compound, previously incubated at 37°C for 30 min, were all survive. The present finding suggests that dialdehyde compound from *Curcuma sp.* rhizome has the antagonistic effect against the lethality of cobra venom.
Keywords: dialdehyde compound, Curcuma sp., Cobra venom, lethality.

Introduction

For many decades, scientific attention has been concentrated on plant natural products which active against snake venom (1). Although the most effective and accepted therapy for snakebite patients is immediate administration of specific antivenom following envenomation, anaphylaxis and serum sickness are frequently resulted (2). The use of traditional medicine in the treatment of poisonous snakebite is still one of the beliefs of people in the tropical countries. However, this type of treatment can either be very useful if it is really effective or extremely dangerous if it has no therapeutic use. Many plants of *Curcuma sp.* (Zingiberaceae) are reputed in Thailand as "snakebite antidote". From our studies (3,4,5), it was found that the rhizomes of these plants contain a common yellow substance proofed to be a dialdehyde compound and beleieved to be an active substance. In the present investigation, both *in vitro* and *in vivo* studies were made to evaluate the efficacy of a dialdehyde compound isolated from *Curcuma sp.* rhizome as the antidote of *Naja kaouthia* venom.

Methodology

1. Fresh venom of *Naja kaouthia* (Fig. 1) in normal saline (9.317 mg protein/ml) was used. A dialdehyde compound isolated from *Curcuma sp.* rhizome (Fig. 2) from Dr.E. Lattmann's laboratory (Aston University, Birmingham, UK.) was dissolved in 95% ethanol (for *in vitro* experiments) or in 5% DMSO (for *in vivo* experiments).

2. Rat phrenic nerve-hemidiaphragm preparations were prepared and submerged in 50 ml Kreb's solution aerated with carbogen and maintained at 37°C. Phrenic nerve was stimulated with the rectangular wave pulse of 0.5 msec duration and frequency of 0.5 Hz.

Muscle contraction was recorded with polygraph. Either the mixture of the dialdehyde compound and normal saline, venom and ethanol or the compound and venom, preincubated at 37°C for 30 min, was added to the bath. The final concentrations of the venom and the dialdehyde compound were expressed as final concentration in the bath. There were at least 6 preparations in each condition tested. The complete inhibition time was recorded and one way ANOVA was used for the statistical test.

3. For the *in vivo* tests, mice were injected subcutaneously with venom (0.75 mg/kg) and 5% DMSO intraperitoneally, the dialdehyde compound (100 mg/kg) orally or intraperitoneally 30 min before the venom or the preincubated mixture (37°C for 30 min) of the compound and venom subcutaneously. There were at least 10 animals in each treatment group. The survival time of the animal were recorded up to 24 hours after receiving the injection of venom. Survival rate in each treatment group were calculated and tested for statistically significant by one way ANOVA, Tukey Test.

Results

***In vitro* experiment:** The venom alone (3.726 μg protein/ml) gradually but completely inhibited the indirectly – evoked twitch and the complete inhibition time was 23.80 ± 2.78 min. The dialdehyde compound (40 mg/ml) appeared to have no effect on the neuromuscular transmission of rat phrenic nerve-hemidiaphragm. The venom preincubated (at 37°C for 30 min before adding to the bath) with the compound at either 4, 10, 20 or 40 mg/ml showed a significant decrease in the toxic effect and resulted in the complete inhibition of the contraction within 67.00 ± 19.88, 84.00 ± 20.45, 127.40 ± 34.33 or 145.00 ± 25.75 min, respectively (Figure 3).

***In vivo* experiment:** Studies in mice showed the inhibitory effect of the compound on lethality of the venom. Figure 4 showed the survival time of the mice treated with different conditions. Mice received subcutaneous injection of venom (0.75 mg protein/kg) were all died and the survival time of the mice was 52.00 ± 5.35 min. Injection with the dialdehyde compound alone (100 mg/kg) showed no effect on the animals and all were survived. Mice received the dialdehyde compound (100 mg/kg) orally or intraperitoneally 30 min before the venom had a survival time of 61.50 ± 5.38 and 88.75 ± 11.15 min, respectively. A significant prolonged of survival time was seen in the latter group. All mice received the preincubated mixture (37°C for 30 min) of the compound and venom, subcutaneously, were survived (up to 24 hours after injection). Death rate of the animals in each treatment group was shown in Figure 5.

Discussion and Conclusion

From the present study, the dialdehyde compound isolated from *Curcuma sp*. rhizome was found to antagonize the inhibitory action of *Naja kaouthia* venom at the neuromuscular junction and also protect mice from lethal dose of venom. However, the effects were clearly observed when the venom was preincubated with the extract. It might be the case that direct binding rather than the interaction at neuromuscular junction be the underlying mechanism. It has been shown earlier that curcumin, one of the curcuminoids from *Curcuma* plants, can interact strongly with biological macromolecules, like serum protein, albumin and hyaluronic acid (6). Accordingly, the immediate application of the dialdehyde compound at sites of snakebites could probably neutralize most of the venom not yet absorbed.

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Figure 1 *Naja kaouthia*

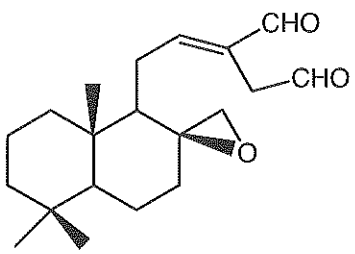

Figure 2 A dialdehyde
(E)-8β, 17-expoxylabd-12-ene-15,16-dial

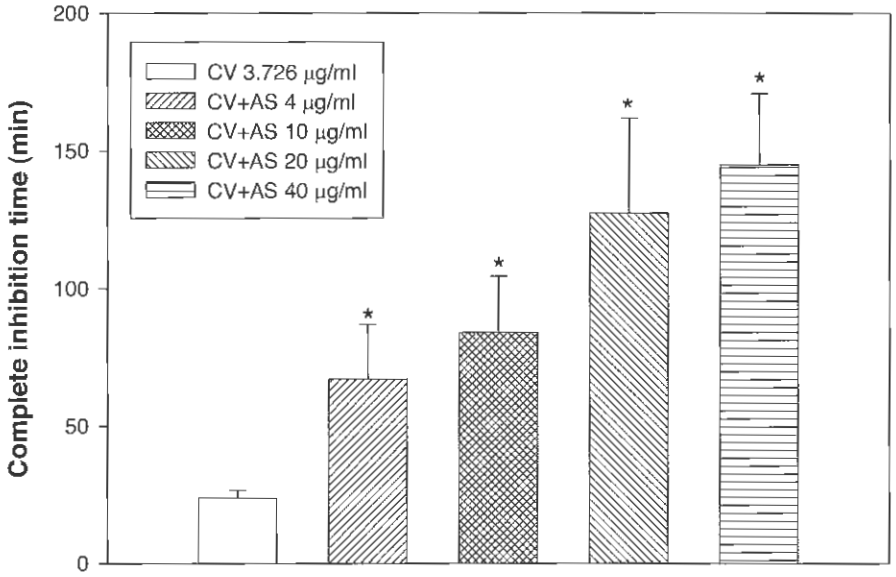

Figure 3 The complete inhibition time of the rat's phrenic nerve-hemidiaphragm preparations after exposure to the venom or the mixture of the venom and the dialdehyde compound. (*p<0.05)

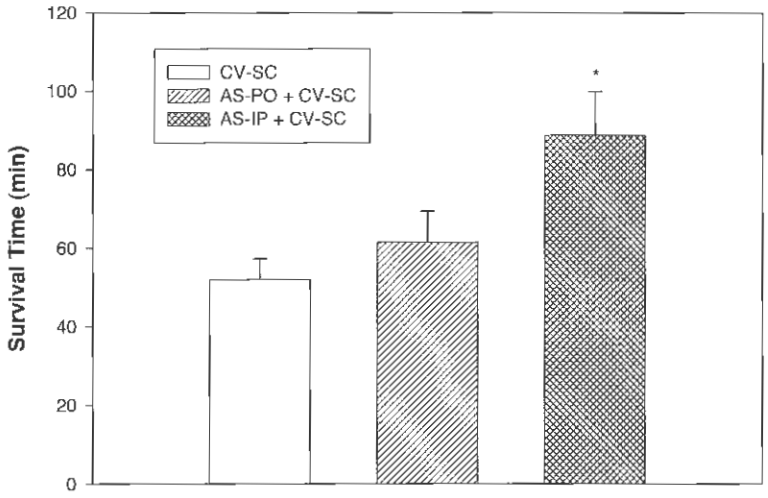

Figure 4 Survival time of mice injected with cobra venom (subcutaneously) alone, or with the dialdehyde compound, either orally or intraperitoneally 30 min before the venom.

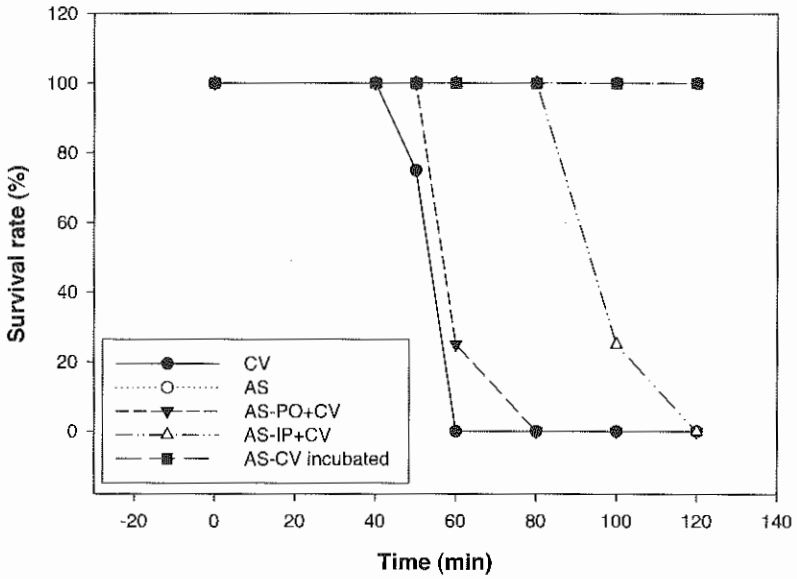

Figure 5 Survival rate (% of the animal in each group) of mice injected with cobra venom (subcutaneously) alone or with the dialdehyde compound, either orally or intraperitoneally 30 min before the venom or the preincubated mixture of the compound and the venom, at 37 ^{0}C for 30 min, subcutaneously.

P05 Antipyretic Effects of Bencha-Loga-Wichien Herbal Drug in Rats.

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Abstract

"Bencha-Loga-Wichien (BLW)" is one of the very famous herbal formula used in Thailand as an antipyretic drug. It is composed of 5 different herbal roots, including *Tiliacora triandra* (Colebr.) Diels (Ya-Nang), *Harrisonia perforata* Merr. (Kon-Ta), *Ficus racemosa* L. (Ma-Due-Au-Thum-Porn), *Clerodendrum indicum* (L.) O. Kuntze (Mai-Tao-Yai-Mom) and *Capparis micracantha* DC. (Ching-Chi). Although listed in "Thailand Essential Herbal Drug List", no scientific data has been shown about the efficacy of "Bencha-Loga-Wichien" as an antipyretic. In this study, "Bencha-Loga-Wichien" powder was orally given to rats at a dose of either 100, 200 or 400 mg/kg. Two hours later, rats were induced to have fever with baker yeast (0.135 g/kg, intraperitoneal injection). It appeared that, at all doses tested, BLW could decrease rectal temperature significantly and highest efficacy was observed at the dose of 200 mg/kg. Each herb at a dose of 40 mg/kg was tested separately for its antipyretic effect and the results showed that *Tiliacora triandra* has the highest and *Clerodendrum indicum* the lowest antipyretic efficacy. No obvious toxic effect of each herb or the formula could be seen. This study suggests that BLW could be used effectively as an antipyretic formula.
Keywords: Bencha-Loga-Wichien, antipyretic effect, yeast-induced fever, rat

Introduction

"Bencha-Loga-Wichien (BLW)" is one of the very famous herbal formula used in Thailand as an antipyretic drug. As powder or tablet, it has been included in "Thailand Herbal Medicine Essential Drug List" as an antipyretic for both children and adults. BLW formula is composed of 5 herbal roots in an equal part by weight, including roots of *Tiliacora triandra* (Colebr.) Diels (Ya-Nang), *Harrisonia perforata* Merr. (Kon-Ta), *Ficus racemosa* L. (Ma-Due-Au-Thum-Porn), *Clerodendrum indicum* (L.) O. Kuntze (Mai-Tao-Yai-Mom) and *Capparis micracantha* DC. (Ching-Chi). Many studies have shown that all those plants contain many alkaloids, sterols and others. The root of *Tiliacora triandra* contains many alkaloids (1) and some of them have antimalarial activities (2). Given the 50% ethanolic extract of Ya-Nang leave to mice, orally or subcutaneously, showed no sign of toxic effect (3). Antipyretic effect of *Ficus racemosa* was recently reported in yeast-induced fever model in rats (4). Antipyretic effect of *Capparis micracantha* root extract was also reported in rabbit (5). No study on antipyretic effect of *Clerodendrum indicum* and *Harrisonia perforata* could be found.

As a formula, BLW is widely used by traditional doctors in Thailand without any scientific data support. Therefore, it is interesting to see whether the drug either as the formula or individual root powder could really reduced fever or not. In this study, yeast-induced fever in rats was used as a studying model.

Methods

Roots of *Tiliacora triandra*, *Harrisonia perforata*, *Ficus racemosa*, *Clerodendrum indicum* and *Capparis micracantha* were collected. Roots were washed, air-dried and further

kept in the oven at 50°C until completely dried. Dried root was powderized and sieved. Fine powder of each root was weighted and kept in the air-tight container. A portion of each powder root was mixed in an equal weight portion and used as a BLW formula. The powder was freshly suspended in normal saline and orally given to the animals and normal saline was used as control. Aspirin at a dose of 150mg/kg orally was used as a positive control. Rectal temperature of each rat was measured many times before starting the experiment to minimize the anxiety state of the animal. Basal rectal temperature of each rat was recorded just before given the drug and rectal temperature was also measured every hour thereafter for another 8 hours. At 2 hours after drug treatment, yeast (0.135 g/kg) suspension was intraperitoneal injected into each rat (6). All animals were used only once. All data were expressed as the rectal temperature changes from the baseline. Statistical analysis was done by using one way ANOVA, Fisher LSD test and $p < 0.05$ was considered statistically significance.

Results

BLW formula: Figure 1 showed the rectal temperature changes at various time after the animals received BLW orally at a dose of either 100, 200 or 400 mg/kg. Not much changes of the rectal temperature could be seen during the first 2 hours before yeast injection. After yeast injection, rats treated with normal saline have increased rectal temperature and the fever was persisted until the end of the experiment. Aspirin could reduce rectal temperature significantly when compared to the control group. BLW showed antipyretic effect with all doses tested, especially at the dose of 200 mg/kg.

Each root: As BLW at 200 mg/kg showed a prominent antipyretic effect, each powdered root at a dose of 40 mg/kg (equal to the part in BLW) was tested in yeast-induced fever model (Figure 2). At 1 and 2 hour after receiving each powdered root orally, rectal temperature of rats received *Tiliacora triandra*, *Ficus racemosa*, and *Capparis micracantha* were significantly lowered than the control. After yeast injection, all powdered root except *Clerodendrum indicum* reduced rats rectaltemperature significantly from the first hour after yeast injection and the effect persisted until the end of the experiment. In *Clerodendrum indicum* treatment group, rectal temperature was lowered than the control group only at T7-8 or 5 and 6 hours after yeast injection.

Discussion and Conclusion

From the present study, BLW was found to have antipyretic effect in yeast-induced fever in rats at dose 100, 200 or 400 mg/kg. It is interesting to note that 200 mg/kg of BLW seemed to be more potent than a dose of 400mg/kg. This might be the case that as a mixture of many compounds, some of them might produce an opposite effect. As a dose increase, the antagonistic effect might be prominent. Each root seemed to be active as an antipyretic drug, except the root of *Clerodendrum indicum* (Mai-Tao-Yai-Mom). However, as the BLW formula, each root might contribute other pharmacological effect which will help ease all the symptoms found in patients. Further study will help clarify the pharmacological action of this interesting Thai herbal medicine and provide scientific support the famous traditional medicine.

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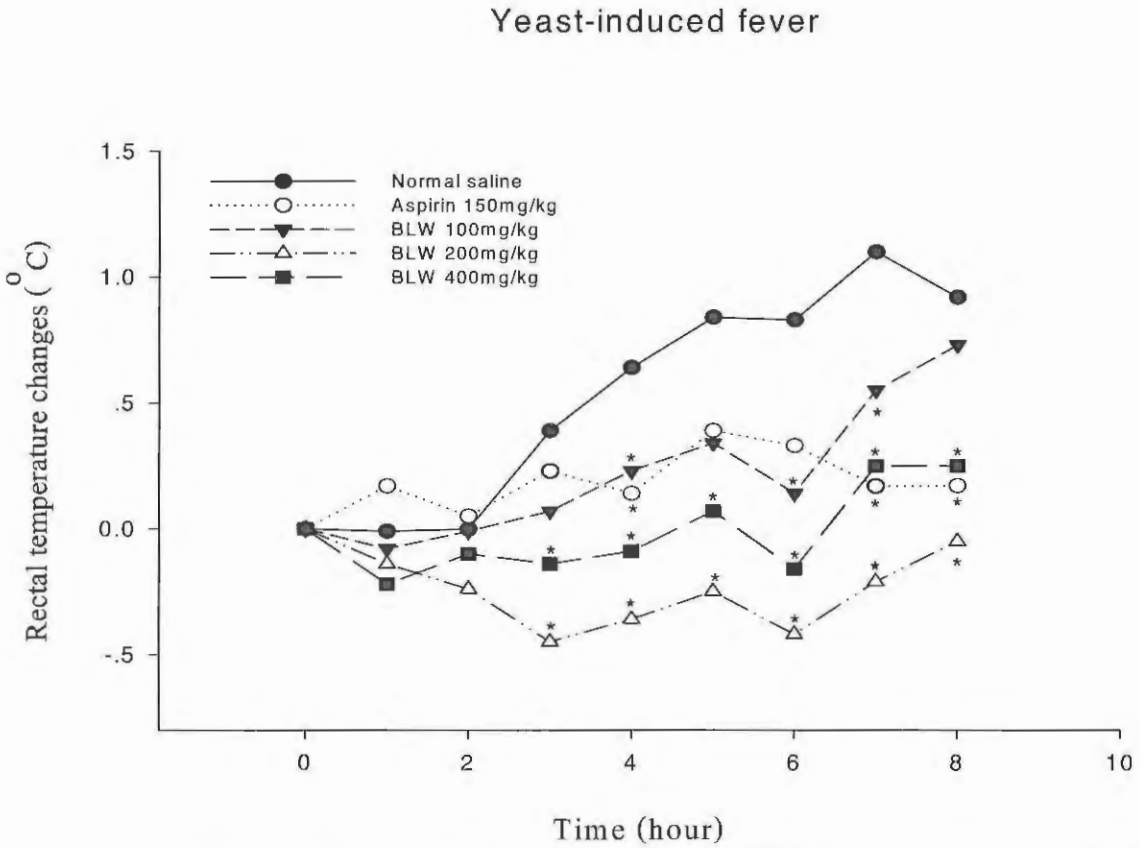

Figure 1 Rectal temperature changes of rats in yeast-induced fever model. Rats were orally fed with "Bencha-Loga-Wichien (BLW)" at a dose of either 100, 200 or 400 mg/kg or normal saline as a control and rectal temperature was measured at every hour thereafter until 8 hours. At 2 hours after BLW administration, yeast was injected intraperitoneally at a dose of 0.135g/kg. (*$p<0.05$ when compared to saline group at the same time)

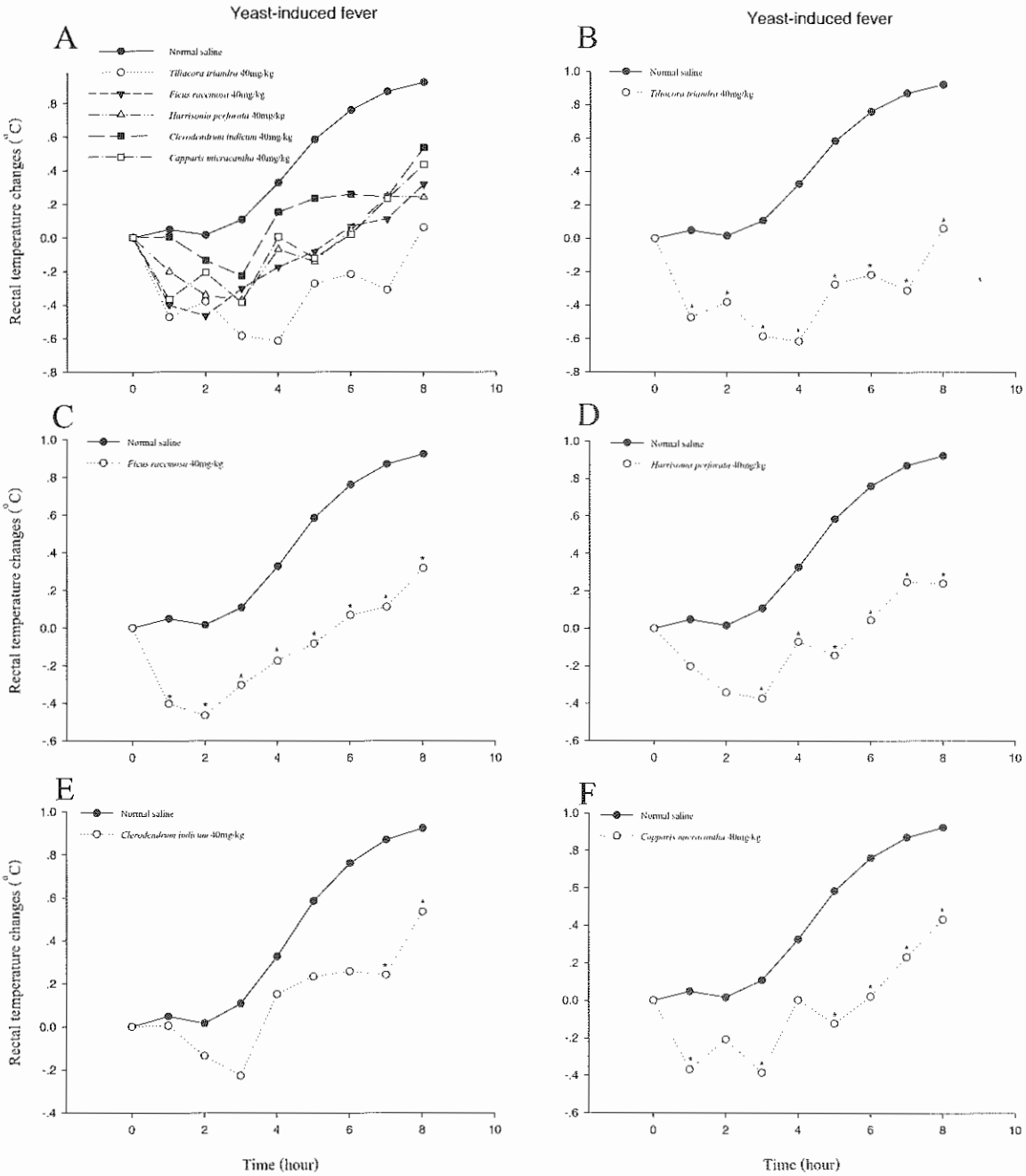

Figure 2 Rectal temperature changes of rats in yeast-induced fever model. Rats were orally fed with normal saline, *Tiliacora triandra*, *Ficus racemosa*, *Harrisonia perforata*, *Clerodendrum indicum* or *Capparis micracantha* in a dose of 40 mg/kg. The experiment was done as described in Fig. 1. A, showed all the treatment conditions. B-F, showed the rectal temperature changes in the animals received each herb. (*$p<0.05$ when compared to saline group at the same time)

P06 Effect of *Curcuma comosa* Powder on Serum Paraoxonase Activities in Cholesterol-Diet Fed Rabbits

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Abstract
Introduction: Serum paraoxonase, PON1 and PON3, is HDL-associated antioxidant enzymes. The role of PON1 and PON3 as anti-atherosclerosis has been clearly demonstrated both *in vitro* and *in vivo*. The activity of PON1 is modulated by various factors including hypolipidemic agents. *Curcuma comosa* Roxb. (Zingiberaceae) is an indigenous plant of Thailand and has been widely used in Thai traditional medicine for treatment of abnormal uterine symptoms. Recently, the hypolipidemic effect of *C. comosa* was extensively investigated.
Objective: We investigated effects of *C. comosa* on PON1 and PON3 activities in cholesterol-diet fed rabbits.
Materials and Methods: Twelve male New Zealand White (NZW) rabbits were treated with 1.0% cholesterol for 1 month and subsequently treated with either 0.5% cholesterol or 0.5% cholesterol combined simvastatin at the dosage of 5 mg/day or 0.5% cholesterol combined *C. comosa* powder at the dosage of 400 mg/kg/day for 3 months.
Results: At 4 months after treatment, lipid parameters and PON1 and PON3 activities were determined. The results showed that *C. comosa* powder significantly decreased levels of total cholesterol and LDL similarly to simvastatin. We found that both *C. comosa* powder and simvastatin did not affect PON1 and PON3 activities.
Conclusion: *C. comosa* powder has hypolipidemic similarly to simvastatin but did not affect PON1 and PON3 activities.
***Keywords*:** Paraoxonase; *Curcuma comosa*; Simvastatin; Cholesterol

Introduction

Serum paraoxonase (PON) consists of two members: PON1 and PON3 which are located adjacent to one another on chromosome 7q21.3-22.1 (1). PON1 and PON3 are expressed primarily in the liver and then secreted into the serum where they are closely associated with HDL (2). Increasing evidences demonstrated that PON1 and PON3 are involved in anti-atherosclerosis. PON1 inhibits copper-induced lipid peroxidation (3). The human PON1 transgenic mice have been found to reduce atherosclerotic lesion (4,5) whereas the PON1 knock out mice accelerated atherosclerosis process and increased lipid peroxidation (6). The information of PON3 is scarcely but has a promising evidence of anti-atherosclerosis properties. Rabbit PON3 is significantly more potent than rabbit PON1 in protecting LDL against oxidative modification (7). Recently, it was found that over-expression of human PON3 in mice reduced atherosclerotic lesion (8). Some clinical data suggest that treatment with hypolipidemic drugs such as simvastatin modulate PON1 activity (9,10). However, at present, it is not known whether simvastatin, might influence PON3 activity.

Curcuma comosa Roxb. is a plant in family Zingiberaceae. It is an indigenous plant of Thailand with a common name in Thai as Waan Chak Mod Look. Rhizomes of *C. comosa* has been used extensively in Thai traditional medicine as an anti-inflammatory agent particularly for the treatment of postpartum uterine bleeding, peri-menopausal bleeding and uterine inflammation. The choleretic effect of *C. comosa* rhizome extract has been recently investigated. It remarkably stimulated bile secretion and enhanced biliary excretion of bile salt and cholesterol which consequently led to a decrease in plasma cholesterol (11).The hypolipidemic effect of *C. comosa* from ethyl acetate extract has been shown to effectively decreased LDL, triglycerides but increased HDL (12,13). The anti-oxidative effect of crude ethanol extract of *C. comosa* has been revealed past year (14). The aim of this study was to investigate the effect of *C. comosa* powder on PON1 and PON3 activities in cholesterol-diet fed rabbits, which was compared with simvastatin, the known medicine using in cardiovascular disease.

Materials & Methods

Materials

Diethyl *p*-nitrophenyl phosphate (paraoxon) and *p*-nitrophenyl butyrate were purchased from Sigma-Aldrich (St.Louis, MO, USA). Phenyl acetate was purchased from Merck (Darmstadt, Germany). Simvastatin was purchased from an accredited drug store (Bangkok, Thailand). *C. comosa* powder was kindly provided by Professor Dr. Apichart Suksamrarn, Faculty of Sciences, Ramkamhaeng University.

Animals and treatment

Twelve male NZW rabbits of body weight between 1.5 – 2.0 kg were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The animals were housed one per cage at the Faculty of Medicine, Srinakharinwirot University, Thailand. All animals were in a controlled humidify room at a constant temperature of 25 ± 2 °C and maintained on a 12-hour alternate light-dark cycle. They were allowed to freely access to food (C.P. Company, Thailand) and drinking water. Prior to the experiment, they were randomly divided into three treatment groups of 4 rabbit each. All treatment groups were given orally with 1.0% cholesterol for 1 month. After 1 month, rabbits in group 1, 2 and 3 were given orally for 3 months with 0.5% cholesterol, 0.5% cholesterol combined simvastatin at the dosage of 5 mg/day and 0.5% cholesterol combined *C. comosa* at the dosage of 400 mg/kg/day, respectively.

Blood sample collection

Blood were collected from 12 hours fasted rabbit at the end of treatment. Plasma were separated and analyzed for lipid profile, liver function and kidney function using auto-analyzer (Hitachi 917) at by Professional Laboratory Management Corp Co., Ltd., Bangkok. Serum were separated and stored at −80 °C until analysis of PON1 and PON3 activities.

Determination of serum PON1 activity

PON1 activity toward paraoxon was measured in 100 mM Tris-HCl buffer pH 8.0 containing 2 mM $CaCl_2$ and 1.1 mM paraoxon at 37 °C (15). The rate of *p*-nitrophenol generation was monitored at 405 nm, and a molar extinction coefficient of 18,700 was used to calculate the enzyme activity. PON1 arylesterase activity was determined in 10 mM Tris-HCl buffer pH 8.0 and 0.9 mM $CaCl_2$ with 1.0 mM phenyl acetate at 37 °C (15). Reaction was monitored at 270 nm, and as extinction coefficient of 1,310 was used for activity calculation.

Determination of serum PON3 activity

PON3 activity was measured in 50 mM Tris-HCl buffer pH 8.0 and 1 mM $CaCl_2$ with 1 mM *p*-nitrophenyl butyrate at 37 °C (16). The rate of of *p*-nitrophenol generation was monitored at 405 nm, and a molar extinction coefficient of 18,700 was used to calculate the enzyme activity.

Statistical analysis

All data were presented as mean ± standard error of the mean (SEM). Differences between groups were analyzed using one-way analysis of variance (ANOVA) by Student-Newman-Keuls and Kruskal-Wallis tests for normally and non-normally distributed parameters, respectively. Changes form baseline outcomes were analyzed using Student's *t*-test. Values of $p<0.05$ were considered to be statistically significant.

Results

Table 1 shows no significant different in the levels of lipid parameters among groups at the baseline. The supplement feeding of cholesterol to rabbits successfully raised the level of lipid parameters at 4 months. The levels of TC, HDL and LDL were highly significant increased at 4 months of treatment in all groups ($p<0.001$) while the level of TG significantly increased at 4 months of treatment in all groups with $p<0.05$ as compared to the levels at baseline.

Table 1 Lipid parameters at baseline and 4 months of the 3 treatment groups

Parameters	Baseline			4 months		
(mg/dl)	Cholesterol	Cholesterol+ Simvastatin	Cholesterol+ *C. comosa*	Cholesterol	Cholesterol+ Simvastatin	Cholesterol+ *C. comosa*
TC	53.8 ± 6.4	54.8 ± 5.4	49.3 ± 5.9	2135.3 ± 169.3**	1575.0 ± 146.6**	1642.8 ± 93.4**
TG	76.3 ± 16.2	57.8 ± 8.1	80.0 ± 7.7	139.3 ± 19.3*	88.0 ± 16.4*	216.3 ± 80.9*
HDL	41.5 ± 6.0	43.0 ± 5.0	38.5 ± 3.9	386.8 ± 17.8**	384.0 ± 83.7**	317.0 ± 17.0**
LDL	14.5 ± 3.5	14.3 ± 2.7	10.5 ± 3.5	2009.3 ± 201.4**	1408.3 ± 103.7**	1463.5 ± 69.0**

Values are mean ± SEM obtained from 4 rabbits. $^{**}p<0.001$ significant difference from baseline. $^{*}p<0.05$ significant difference from baseline. TC=total cholesterol, TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein.

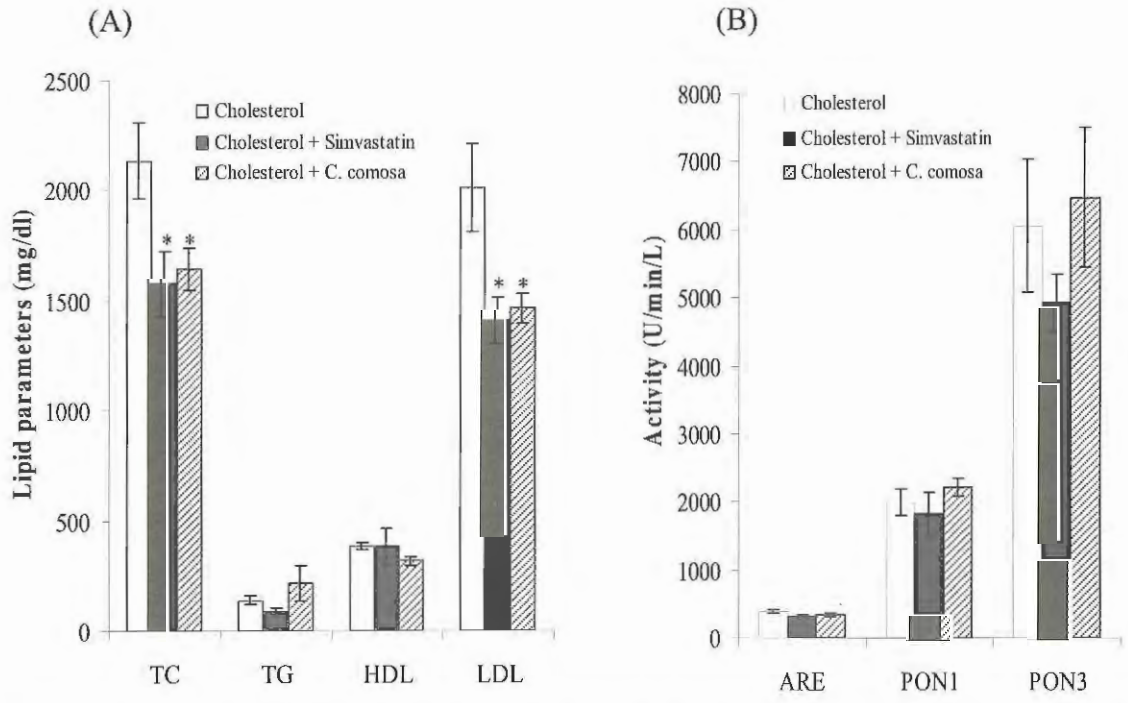

Figure1 Effects of *C. comosa* powder and simvastatin (A) on lipid parameters and (B) on PON1 (using phenyl acetate (ARE) and paraoxon (PON1) as substrates) and PON3 activities at 4 months. Animals were cholesterol-fed, cholesterol-fed with simvastatin and cholesterol-fed with *C. comosa*. Values are mean ± SEM obtained from 4 rabbits. $^{*}p<0.05$ significant difference from cholesterol-fed control.

At 4 months of treatment, both TC and LDL levels in the cholesterol-fed with *C. comosa* and cholesterol-fed with simvastatin groups were significantly decreased than that in the cholesterol-fed control group ($p<0.05$). Remarkably, the decreasing of both TC and LDL levels in the cholesterol-fed with simvastatin group was the same extent as in the cholesterol-fed with *C. comosa*. There were no significant differences in the TG and HDL levels among groups (Fig. 1A).

PON1 activity toward paraoxon and PON3 activities were increased in the cholesterol-fed with *C. comosa* when compared to the cholesterol-fed control, but did not reach statistical significant. The lowest in PON1 activity toward paraoxon and PON3 activities were observed in cholesterol-fed with simvastatin. However, the PON1 activity toward arylesterase was similar in all three groups (Fig. 1B).

Discussion

This study mainly focused on the anti-atherosclerotic effects of *C. comosa* in cholesterol-diet fed rabbit groups by monitoring lipid parameters and serum paraoxonase activity at 4 months of treatment. At 4 months of treatment, the levels of biomarkers for liver function and kidney function were in the reference values in all treatment which indicated that the side effects from the treatment was unlikely occurred (data not shown). Simvastatin decreased TC and LDL levels, but HDL and TG levels remained unchanged. These finding are consistent with the observation found in human (9,10). In our cholesterol-diet fed rabbits, we found that long term treatment with *C. comosa* powder decreased TC and LDL levels as seen in the short term treatment in hypercholesterolemic hamsters (12,13). However, our long term treatment with *C. comosa* powder did not result in the decreased of TG levels. The mechanism of *C. comosa* to lipid parameters is still unknown, it might be associated with interference with the synthesis as well as secretion of lipoprotein into plasma and/or with acceleration of removal of the circulating cholesterol for excretion.

Accumulated data indicated that both PON1 and PON3 are closely associated with HDL and are involved in the prevention of atherosclerosis (7). Hence, the factors influence PON1 activity has been intensively investigated. In addition to reduce plasma lipid, simvastatin with short term treatment has been found to increase the PON1 activity (9,17). In this study, we found that the long term treatment with *C. comosa* powder and simvastatin in cholesterol-diet fed rabbit did not affect PON1 and PON3 activities.

Conclusion

In conclusion, this study demonstrated that long term treatment with *C. comosa* powder decreased total cholesterol and LDL in cholesterol-diet fed rabbits similarly to simvastatin. Both *C. comosa* powder and simvastatin did not affect PON1 and PON3 activities. Our data is limited, thus, true difference between treatment that should present in larger samples might be missed in our study group of 4 rabbits or modulation of PON1 and PON3 might not be associated with the hypolipidemic effect of *C. comosa* powder.

Acknowledgements

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P07 Effects of Hexane extract of *Curcuma comosa* Roxb. on Plaque Formation and Platelet Aggregation in Hypercholesterolemic Rabbits

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Abstract
Introduction: Inflammation plays a crucial role in the development of atherosclerosis. *Curcuma comosa* (*CC*) is an indigenous plant of Thailand which has been traditionally used in folk medicine for the treatment of uterine inflammation. Recent study reported that hexane extract of *CC* contains an estrogenic like action and prevents the release of inflammatory cytokines from activated microglia.
Objective: This study aims to investigate the effects of hexane extracts of *CC* on plasma cholesterol levels, platelet aggregation and plaque formation in high cholesterol-fed rabbits.
Materials and methods: Rabbits were fed with normal rabbit chow, normal rabbit chow + cholesterol, normal rabbit chow +cholesterol + simvastatin, and normal rabbit chow + cholesterol + hexane extract of *CC* for twelve weeks. Plasma cholesterol levels and platelet aggregation were analyzed every 4 weeks. Twelve weeks after the treatment rabbits were sacrificed for histological examination of plaque formation.
Results: The results demonstrated that the hexane extracts of *CC* significantly lowered the cholesterol levels, reduced plaque formation and attenuated platelet aggregation.
Conclusion: Our results suggested that the hexane extracts of *CC* had therapeutic potential for prevention of atherosclerosis.
Keywords: *Curcuma comosa* , Hypercholesterolemic rabbits, Platelet aggregation, Plaque formation

Introduction

Atherosclerosis and related diseases are major causes of death in the industrialized countries. One of the risk factors for the atherosclerosis pathogenesis is hyperlipidemia (1). Platelets are involved in the processes of hemostasis, thrombus formation, inflammatory reactions after an endothelial injury and the development of atherosclerosis (2). Hypercholesterolemia induces a hyperreactive state of the platelets, which enhanced responses to platelet aggregators such as collagen and thromboxane while the antiaggregatory activity of prostacyclin is reduced (3). In addition, the bioactivity of endothelium-derived nitric oxide (NO) can no longer compensate for the atherosclerotic disease process (4).

Curcuma comosa Roxb. is an indigenous plant of Thailand, commonly known as Waan chak mod look. Previous studies demonstrated that *CC* inhibited inflammatory cytokines production (5) and agent possesses anti-inflammatory action prevents atherosclerotic plaque formation (6). Other studies demonstrated the uterotrophic effect and estrogenic activity (7) and hypocholesterolemic effects in hamster (8). Based on these results, we therefore,

investigated the effects of *CC* on plasma cholesterol levels, platelet aggregation and plaque formation in hypercholesterolemic rabbits.

Methods

24 male New Zealand White rabbits initially weighing 1.5-2 kg. were used in this study. The animal study conformed to the Guide to care and Use of Experimental Animal published by the Canadian Council an Animal Care (1993; Vol. 1). The rabbits were randomize-divided into 4 groups. After 2 weeks period of acclimation, the rabbits were exposed to dietary treatment for a period of 12 weeks. The rabbits were fed a commercial diet preparation of Charoen Pokphand Foods Public Company Limited, Thailand.

Rabbits in the first group was fed normal rabbit chow and tap water throughout the experimental period, and served as the control group. Second group was fed a diet containing 0.5% cholesterol served as cholesterol group. Third group was fed a diet containing 0.5% cholesterol and 5 mg/day of simvastatin served as C-simvastatin group. Forth group was fed a diet containing 0.5% cholesterol and 100 mg/kg BW/day of *CC* extract served as C-comosa group. Food and water supplied *ad libitum* throughout the experimental periods. Prior to the beginning of the experiment and at every 4 weeks thereafter, blood samples were drawn by puncture of the marginal ear vein into vacutainers containing 1.2 mgEDTA/mL of whole blood for analysis of cholesterol concentrations. Other blood samples were drawn into siliconized glass tubes containing 3.8% sodium citrate in ratio of 9:1 for determination of platelet aggregation.

At the end of the experimental periods the animals were sacrificed. Segments of the proximal aorta were used for histological examination of the thickening of the aortic plaque formation. The results are expressed as intima/media ratio.

Results

ADP was used to induce platelet aggregation in this study. As shown in figure 1, the responses of platelets to ADP from animals in C-simvastatin and C-comosa treated groups were significantly lesser than that of cholesterol treated group. However, the lower degree of response to ADP was seen in C-comasa group than in C-simvastatin group.

Plasma concentrations of total cholesterol in all groups of rabbits were shown in Table 1. Total plasma cholesterol at baseline in all groups of rabbits was not significant difference. The total cholesterol concentration in control group showed no significant difference throughout the whole experimental period. In contrast, plasma total cholesterol levels in cholesterol group rapidly increased to 927.2 ± 235.4 mg/dL after 4 weeks and continuously increased to 1606.5 ± 96.5 at the end of the experimental period. The total cholesterol levels in C-simvastatin and C-comosa group significantly lowered than cholesterol group. The difference was evident after 4 weeks of treatment. The changes in LDL-C and HDL-C went parallel to plasma total cholesterol in all groups. The level of LDL-C in C-simvastatin group was significantly decreased when compared to cholesterol group at 8 and 12 weeks, for C-comosa group the significant decrease was seen at 8 weeks. The level of HDL-C in C-simvastatin group significantly lowered than the cholesterol group at 12 weeks of treatment but the level of HDL-C in C-comosa group showed no significant difference from the cholesterol group at all treatment periods.

After 12 weeks of the experiment, the intimal thickening of aorta of cholesterol, C-simvastatin and C-comosa groups were significantly increased. However, no intimal thickening was seen in the control group. The intimal thickening in the C-simvastatin and C-comosa groups was significantly lesser than the cholesterol group ($p<0.05$), as shown in figure 2

Discussion

The results in this study demonstrate that the hexane extracts of *C. comosa* Roxb. can decrease platelet aggregation, plasma total cholesterol and reduced the intimal thickening on hypercholesterolemic rabbits. These effects may result from the action of phytoestrogens present in this plant. It has been demonstrated that phytoestrogens have the ability to upregulate the expression of endothelial nitric oxide synthase (eNOS) protein in the endothelial cells of the blood vessels and hence the increase in the production of nitric oxide (NO), causing vasodilation and preventing clotting from blood vessels,(9) and eventually reducing the percent of plaque formation.

Conclusion

The results of this study indicate that the hexane extracts of *Curcuma comosa* Roxb. has a potential in prevention of atherosclerosis in hypercholesterolemic rabbits.

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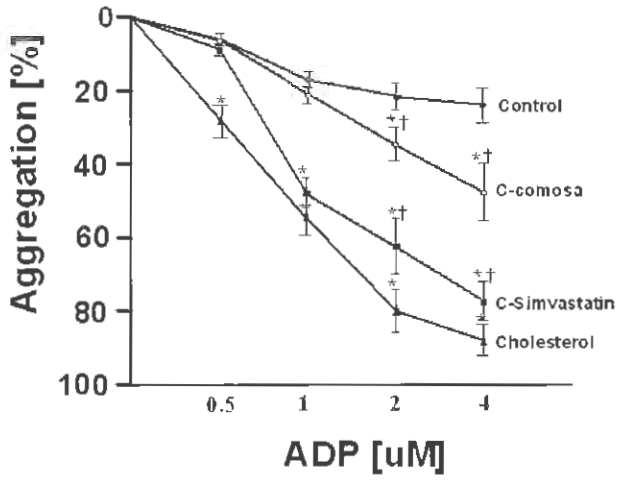

Figure 1 Percent of platelet aggregation induced by various doses of ADP

The percent of platelet aggregation in C-comosa and C-simvastatin groups was significantly lesser than that of the cholesterol group

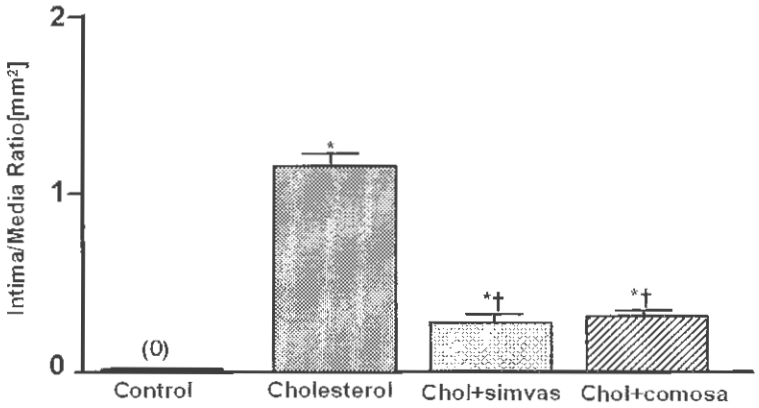

Figure 2 Aortic cross-sectional intima/media ratio in various groups of rabbits

Data are mean ± SEM of the average values of 4 sectional aortic rings from each rabbit (per group n=6) * VS control; † VS cholesterol, $P < 0.05$

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

Group	Time Period (weeks)			
	0	4	8	12
Total Cholesterol (mg/dL)				
Control	41.2±4.2	35.9±3.8	36.7±3.2	37±5.1
Cholesterol	50.00±2.98	927.20±235.44*	1217.60±295.73*	1606.5±96.5*
Chol+Simvastatin	41.75±4.31	618.60±168.94*†	797.40±254.00*†	526.75±201.31*†
Chol+C.*comosa*	52.40±6.01	651.20±205.14*†	709.00±226.77*†	980.4±298.8*†
LDL-Cholesterol (mg/dL)				
Control	10.70±2.2	8.33±2.1	9±1.9	9.5±2.9
Cholesterol	14.20±1.93	873.40±232.13*	1047.00±259.89*	1305.5±87.20*
Chol+Simvastatin	11.75±1.44	584.20±175.08*	745.00±235.26*†	453.5±174.9*†
Chol+C.*comosa*	18.40±3.33	601.20±201.35*	596.80±175.54*†	855.40±264.25*†
HDL-Cholesterol (mg/dL)				
Control	33.67±3.75	28.67±5.24	21.6 ± 2.91	26.7±2.8
Cholesterol	30.80±3.47	220.00±48.39*	310.80±73.82*	331.5±42.51*
Chol+Simvastatin	30.00±4.02	163.00±39.48*	224.6±68.19*	145.75±51.86*†
Chol+C.*comosa*	35.00±3.54	171.00±38.35*	192.4±46.11*	252.6±76.32*

All values are mean ± SEM: * vs control; † $P<0.05$ vs cholesterol; at the same time, $P<0.05$

P08 Neuroprotective Effect of *Curcuma comosa* on Methamphetamine-Induced Microglial Activation and Neurotoxicity

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Abstract

Introduction: Methamphetamine (METH) is a powerful stimulant drug of abuse that is known to be selectively toxic to dopamine (DA) and serotonin (5-HT) terminals in the central nervous system (CNS). The mechanisms underlying this neurotoxicity are not known but oxidative stress has been implicated. Microglia, the brain macrophages, are the principal immune effector cells in the CNS and when activated, they secrete an array of factors that cause neuronal damage. Thus, chemicals that can modulate these syntheses may be beneficial. *Curcuma comosa* is an indigenous plant of Thailand, which has been traditionally and widely used as an anti-inflammatory agent for the treatment of uterine bleeding and uterine inflammation. However, the scientific investigation on its anti-inflammatory activity was limited.

Objective: The aim of this study is to investigate the neuroprotective effect of purified compounds of *C. comosa* fractionated from hexane extract on METH-induced microglial activation and neurotoxicity.

Materials and methods: Human SH-SY5Y neuroblastoma cell line and highly aggressively proliferating immortalized (HAPI) cells, a rat microglia cell line stimulated with METH were used to study the neuroprotective effect of purified compounds of *C. comosa* fractionated from hexane extract.

Results: Our results showed that METH significantly reduces cell viability; tend to increase nitric oxide (NO) production on SH-SY5Y and HAPI cells. Pretreatment of SH-SY5Y cells with *C. comosa* improved the reduction of cell survival induced by METH.

Conclusion: These findings indicated that purified compounds of *C. comosa* fractionated from hexane extract may serve as neuroprotective agents for many neurodegenerative disorders.

Keywords: Neuroprotective, *Curcuma comosa*, Methamphetamine, Microglia

Introduction

Amphetamine derivatives are the most commonly abused drugs. Among them, methamphetamine (METH) is the most popular derivatives used as recreational drugs in developed countries. METH is a neurotoxic illicit drug that may cause long-lasting changes in the dopaminergic pathways of the brain (1), predisposing individuals to parkinsonism (2). Although the mechanisms by which METH causes neurotoxicity are not well understood but

a great deal of interest has centered on oxidative stress as a potential cause (3). In particular, microglia have attracted considerable attention for their roles in mediating damage to the nervous system. Microglia, the brain macrophages, are the principal immune effector cells in the CNS which are responsible for homeostasis regulation and defense against injury. Microglial activation has been shown to play a pivotal role in various neurological disorders (4). Proinflammatory substances produced by microglia such as cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO) result in neuronal damage and further exacerbate the neuroinflammatory states of the diseases (5). Thus, chemicals that can modulate these syntheses may be beneficial. Estrogen and phytoestrogens have been shown to be neuroprotective in several neurotoxicity models (6, 7); however, their effects in microglia have not been well established.

Curcuma comosa Roxb. (Zingiberaceae), commonly knows as *Waan chak mod look* in Thai, is an indigenous plant of Thailand which has been generally used in folk medicine as an anti-inflammatory agent for the treatment of postpartum uterine bleeding, peri-menopausal bleeding, and uterine inflammation. Despite its long-term and wide use, there is scant scientific evidence on the anti-inflammatory activity of the plant. The result from previous study demonstrated the estrogenic-like activity of the plant and they suggested the presence of phytoestrogens in the hexane extract of this plant (8). It has been reported that phytoestrogens possess anti-inflammatory and neuroprotective activities in the CNS (9). Recent study has demonstrated that hexane extract from the rhizome of *C. comosa* suppressed NO/iNOS synthesis, MCP-1 and IL-6 expression in HAPI cells in LPS-induced microgliosis (10). However, there is no scientific investigation of the activity of purified compounds from *C. comosa* extract especially in the brain.

In the present study we investigated the neuroprotective effects of purified compounds of *C. comosa* fractionated from hexane extract on METH-induced microglial activation and neurotoxicity by using human SH-SY5Y neuroblastoma cell line and highly aggressively proliferating immortalized (HAPI) cells, a rat microglia cell line.

Materials and Methods

Cell cultures

Human SH-SY5Y neuroblastoma cell line were maintained in a mixture of Eagle's Minimal Essential Medium (MEM) and Nutrient Mixture Ham's F-12 medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin, and 100 μg/ml streptomycin. Highly aggressive proliferating immortalized (HAPI) microglial cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% heat-inactivated FBS. In all experiments, cells were left to acclimate for 24 hr before any treatments. The purified compounds of *C. comosa* fractionated from hexane extract were always added 2 hr prior to METH.

MTT assay

The number of viable cells were determined by the ability of mitochondria to convert MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan dye. SH-SY5Y and HAPI cells were cultured onto 96-well plate at a density of 2 x 10^4 cells/well overnight. The cells were then treated with various reagents according to the experimental design. After 24 hr the medium was removed and 50 μl of 1 mg/ml MTT in Hank's balanced salt solution (HBSS) was added to each well and further incubated for 4 hr in a humidified atmosphere at 37 °C, 5% CO_2. MTT was removed and cells were lysed with 100 μl DMSO, then the absorbance was measured at 570 nm and at a reference wavelength of 620 nm on a microplate reader.

Griess assay

To determine NO produced from METH-treated cells, SH-SY5Y and HAPI (5 x 10^5 cells/well) were plated onto 6-well plate and treated with METH. At 24 hr after activation, the cells culture supernatant from each sample was collected and the equal volume of Griess reagent was added. After 15-min incubation at room temperature, optical density at 545 nm of the sample was determined by an automated microplate reader. Sodium nitrite, diluted in culture media at 0 to 100 μM concentration, was used as standard curve.

Statistical analysis

Data are presented as mean ± S.E.M. from three or more independent experiments. Statistical comparison between different treatments was done by one-way ANOVA with Tukey's multiple comparison post-test using SPSS program version 15. Differences were considered significant at $p<0.05$.

Results

Effects of METH and *C. comosa* on cell viability

SH-SY5Y cells were treated with increasing concentrations of METH ranging from 0.1 to 3 mM for 24 and 48 hr and cell viability was determined. METH produced a dose- and time-dependent decrease in cell viability. At 24 hr cell viability decreased to 92.6 ± 0.3, 84.2 ± 2.5, 80.3 ± 1.9, 73.3 ± 1.6, 54.3 ± 3.9% of the control values with 0.1, 0.5, 1, 2 and 3 mM METH treatment, respectively. At 48 hr cell viability decreased to 81.3 ± 3.2, 74.7 ± 4.1, 71.8 ± 4.9, 60.9 ± 4.4, 29 ± 2.5% of the control values with 0.1, 0.5, 1, 2 and 3 mM METH treatment, respectively, which demonstrated that the neurotoxic effect of METH on dopaminergic neurons was mediated in a dose- and time-dependent manner.

HAPI cells were treated with increasing concentrations of METH ranging from 0.1 to 3 mM for 24 and cell viability was determined. MTT assay showed that METH appeared to decrease cell survival concentration-dependently. At 24 hr cell viability decreased to 89.4 ± 0.6, 81.6 ± 0.9, 77.8 ± 1.4, 70.7 ± 1.6, 55.9 ± 1.6% of the control values with 0.1, 0.5, 1, 2 and 3 mM METH treatment, respectively, indicating the neurotoxic effect of METH.

The cytotoxic effect of purified compounds of *C. comosa* fractionated from hexane extract on SH-SY5Y and HAPI cells was evaluated. Both cells were treated with various concentrations of *C. comosa* ranging from 0.001 μM to 100 μM for 24 hr and were subject to MTT assay. The purified compounds of *C. comosa* fractionated from hexane extract at concentrations from 0.001 μM to 10 μM had no effect on cell survival. However, the viability of the cells was remarkably reduced to only 1% of control when 100 μM of purified compounds of *C. comosa* fractionated from hexane extract was used.

In order to determine whether *C. comosa* provides neuroprotection, SH-SY5Y cells were exposed to 2.5 mM METH with or without pretreatment with *C. comosa*. The viability of cells incubated with 2.5 mM METH for 24 hr was 65.9 ± 1.6% of the control value. The viability of cells incubated with *C. comosa* at 100 nM for 2 hr prior treatment with 2.5 mM METH for additional 24 hr was 85.5 ± 2.0% of the control value. Pretreatment with *C. comosa* for 2 hr had no effect on cell viability when compared with control untreated cells.

Effect of METH on NO productiou

SH-SY5Y and HAPI cells were incubated with METH from 0.1 mM to 3 mM for 24 hr and the levels of NO in the culture media were determined with Griess assay. METH at low concentrations (0.1, 0.5 and 1 mM) did not affect NO production but at high concentrations of METH (2 and 3 mM) tend to increase NO production.

Discussion

The present study has shown that METH induces SH-SY5Y cell death in a concentration and time-dependent manner, whereas METH inducing HAPI cell death is only

concentration-dependent. However, pretreatment of SH-SY5Y cells with purified compounds of *C. comosa* fractionated from hexane extract reduced the loss of cell viability caused by METH treatment. These effects may result from the action of phytoestrogens present in this plant. The study of *C. comosa* and its role in neuroprotection is still in a very early stage. Further studies are needed to explore mechanisms of *C. comosa* that confer these neuroprotective effects in microglia and neurons.

Conclusion

The results of this study indicate that purified compounds of *C. comosa* fractionated from hexane extract may serve as neuroprotective agents for many neurodegenerative diseases.

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P09 Effects of Purified Natural and Synthesized Compounds from *Curcuma longa* on Neurotoxicity in SH-SY5Y cell line.

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Abstract

Introduction: It has been known that methamphetamine (METH) induces the formation of free radicals and other inflammatory factors causing the damage of monoaminergic system, especially the dopaminergic pathway of CNS both in vitro model, experimental animals and also postmortem striatum of chronic METH users. Curcumin, the active component in turmeric, has been shown to possess anti-oxidant, anti-inflammatory and anti-carcinogenic activities by reducing the formation of free radicals and the production of apoptotic and inflammatory factors and also modulating transcription factors such as ROS, NO, ILs, BAX, nNOS, COX-2 and NF-κB, which play a major role in neurodegeneration.

Objective: The present study was aimed to evaluate the effects of purified natural and synthesized compounds from *Curcuma longa,* which are curcumin I, II, III and AS-YS 001-004, on survival of SH-SY5Y neuroblastoma cell line in order to further study the protective effect of those compounds on METH-induced neurotoxicity.

Materials and methods: SH-SY5Y cells were treated with various concentrations of purified natural and synthesized compounds from *C. longa* and also METH, and the number of cell viability was determined by MTT reduction assay.

Results: These finding showed that METH-induced neurotoxicity in a concentration and time dependent manner. Moreover, at concentration 0.001-0.1 μM of curcumin I, II, II and AS-YS 002-004 could preserve cell viability, suggesting that might be a suitable concentration for preventing METH-induced neurotoxicity.

Conclusion: The purified natural and synthesized compounds from *C. longa* might be used as compound to protect neuron.

Keyword: Methamphetamine (METH)/ *Curcuma longa* (curcumin)

Iutroduction

Parkinson's disease (PD) is an idiopathic disorder whose signs include tremor, hypokinesia, muscular rigidity and loss of postural reflexes (1). Parkinsonism can also be seen after exposure to a number of toxins (2).This is a slow, progressive disease and is characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra (SN) and intraneuronal cytoplasmic inclusions called Lewy Bodies (3). A number of important results have been accumulated from pathological and pharmacological studies on PD and from animal or in vitro studies using dopaminergic neurotoxins which cause parkinsonism in animals.

Methamphetamine (METH) is a well known drug of abuse and neurotoxin that may cause long-lasting changes in the CNS dopaminergic pathway(4). METH is amphetamine molecule with an additional methyl group attached to its nitrogen amine group. The additional methyl group results in METH having a slightly higher pKa value than AMP. This should mean that METH has a greater speed of absorption (5). Therefore, it is closely related chemically to amphetamine, but the central nervous system effects of methamphetamine are greater. It causes neurotoxicity in rodents and non-human primates by producing long-term depletion of

dopamine (DA) and its metabolites (6, 7), decreasing the number of high affinity DA uptake sites (8) and decreasing the activity of tyrosine hydroxylase (TH) in striatum (9). Moreover, DA, TH, and the DA transporter were reduced in the postmortem striatum of chronic METH users (10). METH induced DA release from the vesicle to the extracellular space by means of reverse transport (11) along with formation of free radicals such as reactive oxygen species (ROS) (12) and reactive nitrogen species (RNS) (13), resulting in the formation of peroxynitrite, a highly reactive molecule causing neurotoxicity, is thought to one of the main mechanisms involved in METH-induced neurotoxicity. Thus, it is considered as one of the model for drug-induced dopaminergic and neural toxicity.

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the principle yellow active substances isolated from turmeric, a powder forms of *Curcuma longa* Linn, is widely used for the coloring of food. The major natural curcumin present in turmeric contains diferuloymethane (curcumin I), demethoxycurcumin (curcumin II) and bisdemethoxycurcumin (curcumin III) approximately 77, 17 and 3%, respectively (14). Moreover, one of curcumin's major metabolites is tetrahydrocurcumin or THC. A large number of evidence has reported that curcumin possesses an anti-oxidant (15), anti-inflammatory (16) and anti-carcinogenic activity, it therefore is considered to be used as the supplement compound for its therapeutic purpose.

Methods

Substrates The purified natural and synthesized compounds from *C. longa*, diferuloymethane (curcumin I), demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III) and AS-YS 001-004, was provided by Professor Dr. Apichat Suksamrarn, Department of chemistry, Ramkhamheang University.

Cell culture SH-SY5Y cells from a human dopaminergic neuroblastoma cell line were purchased from American Type Culture Collection (ATCC), (Manassas, VA). They were grown in a medium containing MEM supplemented with 10% heat-inactivated FBS, 100 units/ml of penicillin, and 100 µg/ml streptomycin at 37°C under a humidified 5% CO_2 and 95% air atmosphere. At confluence, the cells were seeded onto culture plates for further experiment.

MTT reduction assay The number of viable cells were determined by the mitochondrial conversion of yellow MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan dye. SH-SY5Y cells were seeded onto 96-well cell culture plate at a density of 2 x 10^4 cells/well. Cells were treated with various concentrations of purified natural and synthesized compounds from *C. longa* for 24 in order to investigate the effect on cell viability. All results will be taken for further study the protective effect of these compounds on METH-induced neurotoxicity. The cellular reduction of MTT which represents metabolic activity and viability is expressed as the percent absorbance of treated cells compared with the absorbance of control cells.

Data analysis Data are presented as mean ± S.E.M. from three independent experiments. Statistical comparison between different treatments was done by one-way ANOVA with Tukey's multiple comparison post-test using GraphPad Prism program versions 5. The significance was taken when p values were 0.05 or less.

Results

1. Effects of METH–induced reduction in cell viability in SH-SY5Y cells.

1.1 Concentration and time-dependent effects of METH on cell viability

To evaluate the effect of METH-induced neurotoxicity. SH-SY5Y cells were treated with increasing concentrations of METH ranging from 0.1 to 3 mM for 12, 24, and 48 h and cell viability was determined. MTT assay showed that METH-induced SH-SY5Y cell death in a

concentration and time-dependent manner. METH at a concentration of 3 mM decreased cell survival to 75.86%, 54.27%, and 28.94% compared to control for 12, 24, and 48 h, respectively.

2. Effects of purified natural and synthesized compounds from *C. longa* on cell viability in SH-SY5Y cells

2.1 To evaluate the effect of purified natural compounds from *C. longa* on cell viability. SH-SY5Y cells were treated with increasing concentrations of purified natural compounds from *C. longa* ranging from 0.001 to 100 μM 24 h and cell viability was determined. At concentration ranging from 0.001—0.1μM of curcumin I, II, II and AS-YS 002-004 could preserve cell viability more than 90%.

Discussion

In this study, we would like to evaluate the effect of purified natural and synthesized compounds from *C. longa* in order to further study the neuroprotective of these compounds on METH-induced neurotoxicity. The results showed that METH significantly induced cell cytotoxicity in a concentration and time dependent manner. At 0.001- 0.1 μM of curcumin I, II, III and AS-YS 002-004 could preserve the number of cell survival. Moreover, more than 0.1 μM of AS-YS 001 could significantly increase the number of cell death. It has been suggested that this compound might be toxic to neuron and unsuitable for further study.

In conclusion, METH can be used as a neurotoxin and experimental model for inducing neurotoxicity of dopaminergic CNS system. At 0.001-0.1 μM of purified natural and synthesized compounds from *C .longa* except AS-YS 001 might be a suitable concentration for further study the neuroprotective effects of these compounds on METH-induced neurotoxicity.

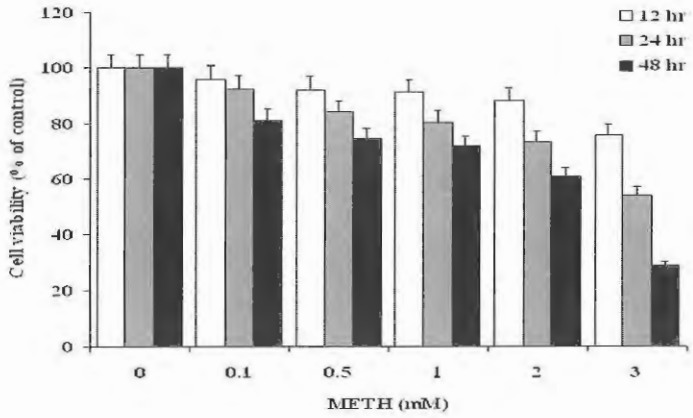

Figure 1. Concentration- and time-dependent effects of METH on cell viability in SH-SY5Y cells. SH-SY5Y cells (2 x 10^4 cells/well) were treated with various concentrations of METH (0.1, 0.5, 1, 2, and 3 mM) for 12, 24, and 48 h. Cell viability was assessed by the MTT assay and is presented as percent of untreated controls. Values represent the mean ± SEM of three separate determinations.

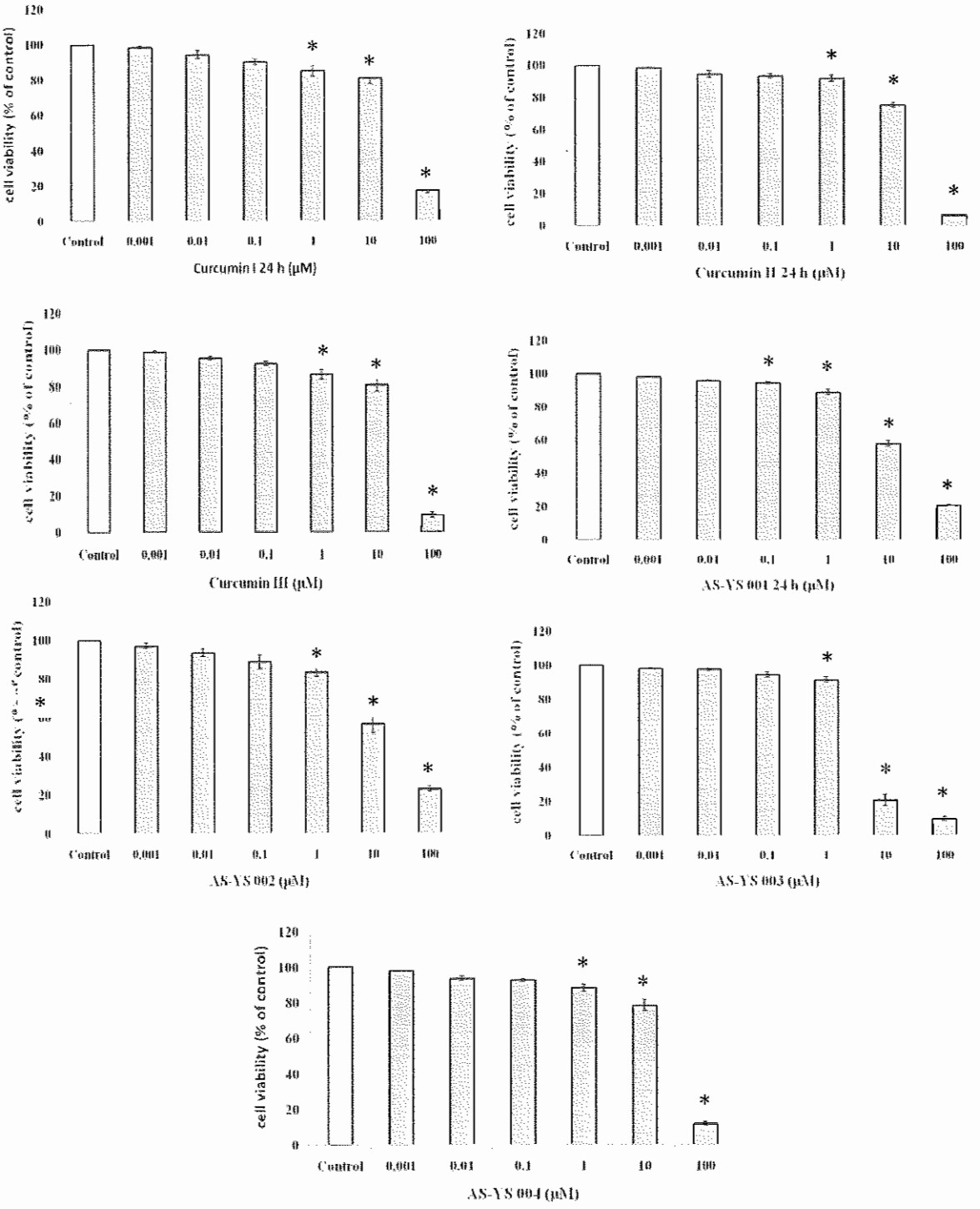

Figure 2. Effect of purified synthesized compounds from *C. longa* on cell viability in SH-SY5Y cells. SH-SY5Y cells (2 x 10^4 cells/well) were treated with various concentrations of purified natural and synthesized compounds from *C. longa* (0.001, 0.01, 0.1, 1, 10 and 100 μM) for 24 h. Cell viability was assessed by the MTT assay and is presented as percent of untreated controls. Values represent the mean ± SEM of three separate determinations. *P<0.05 significance compare with untreated controls

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P10 Vasoprotective Effects of *Pueraria mirifica* Extract in Ovariectomized Rats.

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Abstract

Introduction: *Pueraria mirifica* Airy Shaw and Suvatabandhu, a phytoestrogens containing herb, has been used as traditional medicine and exhibits comparable effects to estrogen. Phytoestrogens supplementation is an alternative way of hormone replacement therapy (HRT) in post-menopausal women, in particular to preserves the protective effects on cardiovascular system and minimizes the side effects in long term estrogen treatment.

Objective: This experiment was designed to determine the vasoprotective effects of *P. mirifica* in ovariectomized rats.

Materials and methods: Thirty two female Wistar albino rats were randomly assigned into 4 groups. Group 1, 2 and 3 were undergone bilateral ovariectomy and orally administered with *P. mirifica* 100 mg/kg/day (OVX+*P. mirifica*), subcutaneously injected of estradiol valerate 300 μg/kg/week (OVX+Estrogen) and distilled water fed (OVX), respectively. Group 4 was sham operated and treated with distilled water (Sham). All of them were treated for 42 consecutive days. At the end of treatments, blood samples were obtained by cardiac puncture for determination of nitric oxide (NO) and lipid parameters (total cholesterol, HDL-C, LDL-C and triglyceride). Descending thoracic aortas were isolated for vascular function measurements and histopathological studies.

Results: Acetylcholine-mediated endothelium-dependent vascular relaxation was significantly impaired in OVX group ($p < 0.05$ vs. normal vessel), but restored in the OVX+*P. mirifica* and OVX+Estrogen groups. Microscopic examinations of thoracic aortas in the OVX+*P. mirifica* and the OVX+estrogen groups showed the lesser degree of medial smooth muscle and endothelial cell degenerations. Compared to the OVX group, treatment with *P. mirifica* resulted in significantly increased in NO production.

Conclusion: These results indicate that treatment with the *P. mirifica* preparation preserves endothelial vasodilator function and vascular structures in ovariectomized rats.

Keywords: *Pueraria mirifica*, Vascular function, endothelial cell, Ovariectomized rat

Introduction

Oophorectomized and post-menopausal women suffer from many menopausal symptoms. Accordingly, important abnormality including cardiovascular diseases (CVDs) has been reported (1). The losses of protective effects of endogenous estrogen on cardiovascular system are considered to be the main cause of this abnormality (2). Estrogen replacement therapy (ERT) is an effective method to prevent menopausal symptoms and restore the protective effects on cardiovascular system (3). However, many serious side effects in long term ERT used including cancerous events and thromboembolic events have been reported (4).

Pueraria mirifica (white "Kwao-Keur") is an indigenous herb of Thailand which possesses estrogenic activity. The phytoestrogens that possess the highest estrogenic activity in tuberous root of *P. mirifica* are chromene derivative compounds, deoxymiroestrol and

miroestrol (5). Despite evidences linking phytoestrogens of *P. mirifica* exhibit strong estrogenic activities, beneficial effects of *P. mirifica* preparation using as ERT in particular on vascular functions and vascular structures remain unclearly defined.

Therefore, the objcctives of this study were to determine the subchronic effects of *P. mirifica* preparation on vascular functions in isolated thoracic aorta preparations in ovariectomized rat model. In parallel sets of experiment, the effects of *P. mirifica* on cardiovascular-related blood biochemistry parameters were also examined.

Methods

Thirty two female Wistar albino rats were randomly divided into 4 groups. Group 1, 2 and 3 were operated to remove both of bilateral ovaries (ovariectomy) and orally administercd with *P. mirifica* 100 mg/kg/day (OVX+ *P. mirifica*), subcutaneous injected of estradiol valerate 300 µg/kg/week (OVX+Estrogen) and distilled water fed (OVX), respectively. Group 4 was undergone laparotomy operation and treated with distilled water (Sham). All of them were treated for 42 consecutive days.

At the end of treatments, blood samples were collected by cardiac puncture for determination of cardiovascular-related blood biochemistry parameters including nitric oxide (NO) and lipid parameters (total cholesterol, HDL-C, LDL-C and triglyceride). Descending thoracic aortas were isolated for histopathological studies. Vascular functions were measured as isolated thoracic aorta preparations including vasocontraction response to noradrenaline (NA), vasorelaxation response to acctylcholine (Ach) and sodium nitroprusside (SNP) in NA-precontracted aortic rings.

Results

Compared to the Sham group, Ach-mediated endothelium-dependent vascular relaxation in OVX group was significantly impaired, but restored in OVX+ *P. mirifica* and OVX+Estrogen groups. The percentages of vascular relaxation in OVX+ *P. mirifica* were significantly higher than those in OVX group at 10^{-7} to 10^{-4} M of Ach concentration (Figure 1A). While the significant differences of SNP-mediated endothelium-independent vascular relaxation and NA (10^{-9} to 10^{-5} M)-induced vascular contraction among the group were not identified (Figure 1B and 1C).

The plasma levels of NO in OVX+ *P. mirifica*, OVX+Estrogen and Sham group were not significantly different from each other. However, the plasma NO levels of these groups were significantly higher than the OVX group (Figure 2A). The OVX group showed a considerable rise in all lipid parameters compared with that in the Sham group which significant different in total cholesterol and LDL-C levels. The plasma TG level in OVX+ *P. mirifica* was lower than that in OVX group, but not significant different (Figure 2B). The atherogenic index, (total cholesterol – HDL-C) / HDL-C ratio, of OVX+ *P. mirifica* and OVX+Estrogen were lower than the OVX group but not significant different (Figure 2C).

Microscopic studies of descending thoracic aortas in the OVX+*P. mirifica* and the OVX+Estrogen groups showed the lesser degree of medial smooth muscle and endothelial cell degenerations, compared with OVX group (Figure 3).

Discussion

The results of this present study demonstrated the improvement of endothelium-dependent vascular relaxation due to the beneficial effects on lipid profiles and endothelial cell preservation in *P. mirifica* treated ovariectomized rats. Improvement of endothelial dysfunction which was correlated with an increase of NO production in blood was considered to be the results of the antioxidant effects of *P. mirifica* in comparison with estrogen. These

are involved in atherosclerosis prevention and restoration of vascular smooth muscle co-ordination in arterial wall and balance in adrenergic response of vascular contraction.

Conclusion

The results in the present studies provided the evidences that *P. mirifica* may exert estrogen-like effects. The data supports the beneficial effects and knowledge in using of *P. mirifica* in Thai traditional medicine. The efficacy of using *P. mirifica* as an alternative hormone replacement therapy (HRT) in postmenopausal women is also demonstrated. In order to clarify the exact mechanisms of its protective effects on vascular in aging women, further studies of *P. mirifica* effects on estrogen receptors in various tissues as well as its antioxidant properties should be examined.

Acknowledgement

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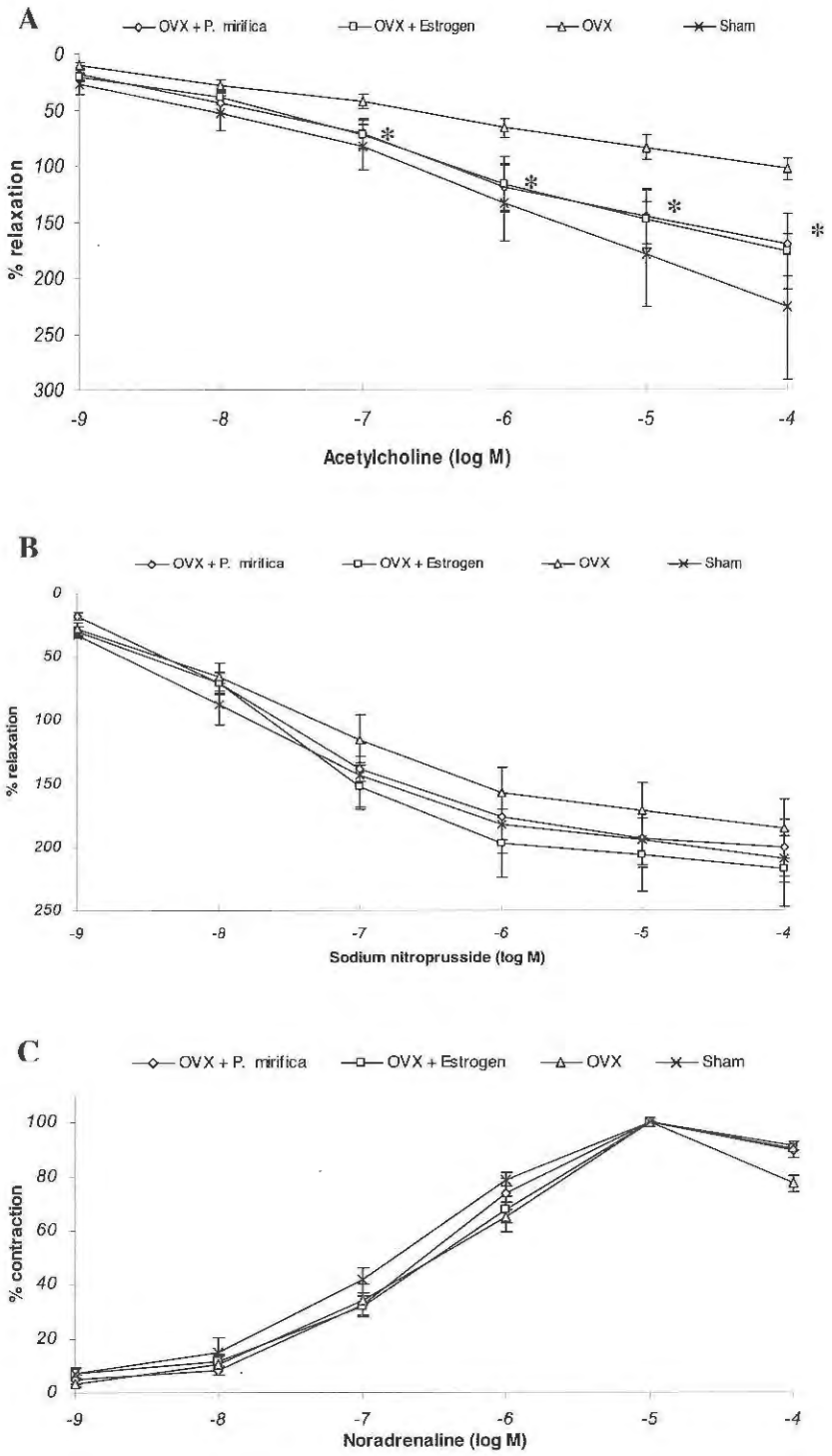

Figure 1 Vascular responses in isolated rat thoracic aortas from non-OVX control (Sham), OVX, OVX+Estrogen and OVX+*P. mirifica* (mean ± S.E.M., * $p < 0.05$ vs. OVX).

A: Vasorelaxation response to acetylcholine (Ach) in NA-precontracted aortic rings

B: Vasorelaxation response to sodium nitroprusside (SNP) in NA-precontracted aortic rings

C: Vasocontraction response to noradrenaline (NA)

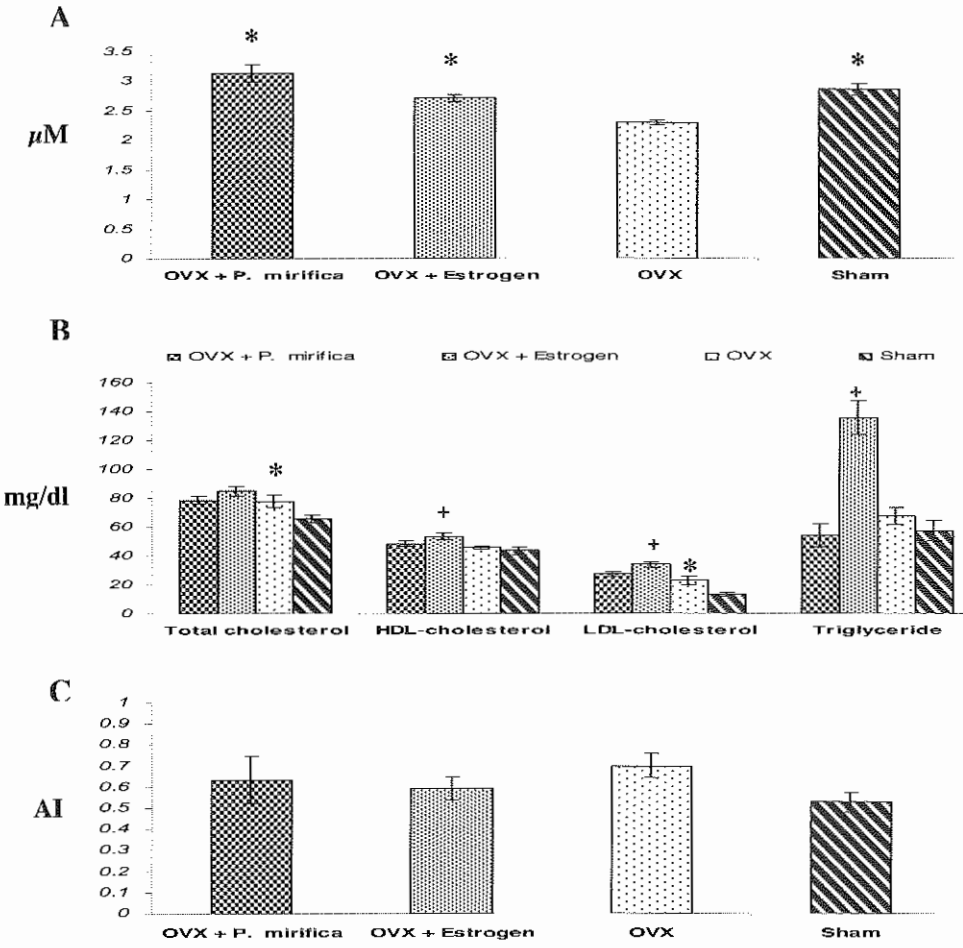

Figure 2 Cardiovascular-related blood biochemistry parameters from non-OVX control (Sham), OVX, OVX+Estrogen and OVX+*P. mirifica* (mean ± S.E.M., * $p < 0.05$ vs. OVX, $^{+}$ $p < 0.05$ vs. Sham).

A: Plasma nitric oxide (NO) production

B: Plasma level of lipid profiles (Total cholesterol, HDL-C, LDL-C and Triglyceride)

C: Atherogenic Index [(total cholesterol – HDL-C) / HDL-C ratio]

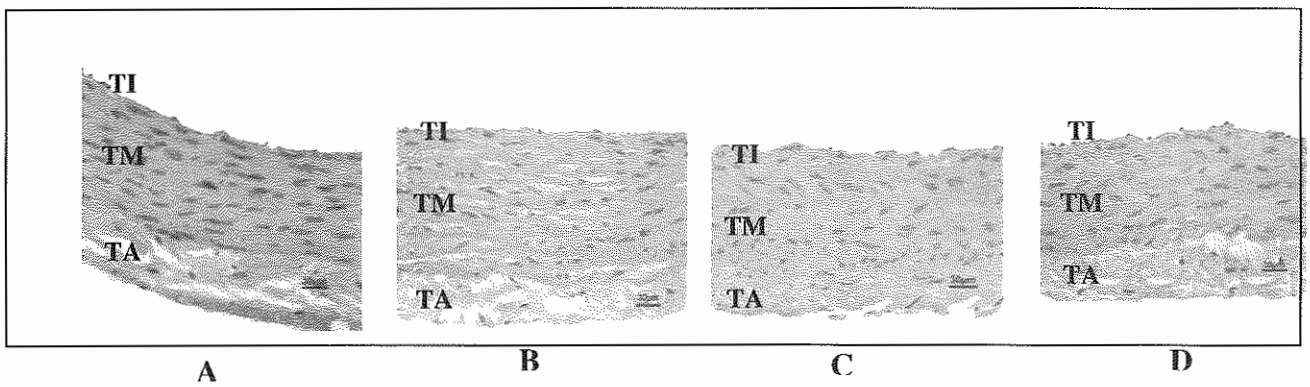

Figure 3 Histopathological figures of descending thoracic aortas of the study rats.

TI: tunica intima, **TM**: tunica media, **TA**: tunica adventitia

A: non-OVX control (Sham), **B**: OVX, **C**: OVX+Estrogen, **D**: OVX+*P. mirifica*

P11 Radical Scavenging and Neuroprotective Activities from Extracts of *Centella asiatica*

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Abstract

Oxidative stress resulting from the toxic effects of free radicals on tissue plays an important role in the pathogenesis of various neurodegenerative diseases such as cerebral ischemia, Parkinson's disease and Alzheimer's disease. Natural antioxidants can scavenge free radicals and prevent the human body from aging by reducing oxidative stress. The present study was performed to evaluate antioxidative and neuroprotective properties of *Centella asiatica* Urban (CA). Radical scavenging effects were evaluated by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. The cytotoxic and neuroprotective properties were evaluated on neuroblastoma NG108-15 cells. Oxidative stress was induced by hydrogen peroxide (H_2O_2). In the DPPH assay, the radical scavenging activity of water was higher than the methanol and chloroform extracts (IC_{50} = 62, 84 and 243 μg/ml, respectively). In cytotoxic experiment, water and chloroform extracts (1 mg/ml) caused cell death. In neuroprotective study, when being added simultaneously with H_2O_2 (150 μM), the methanol extracts (10-100 μg/ml) effectively protected cells from oxidative damage. Therefore, the present results indicated that methanol extracts from CA acts as antioxidants and neuroprotective agents against oxidative damage. Thus the methanol extract from CA should be further investigated to be used in prevention and treatment of disorders which results from oxidative stress.

Keywords: Neurotoprotective, antioxidant, *Centella asiatica* , neuroblastoma NG108-15 cells, oxidative stress

Introductiou

In recent years, there has been an increased interest in the application of antioxidants for medical treatment as more information links the development of human diseases to oxidative stress. Oxidative stress, which results from an imbalance between the antioxidant defense system and the formation of reactive oxygen species (ROS), may damage important membrane lipids, proteins, DNA and carbohydrates. In neurology, it is generally agreed that ROS are one of the major and important factors contributing to degenerative brain diseases such as Alzheimer's, Parkinson's disease, epilepsy, and stroke(1). A variety of plant products that have potent antioxidants properties have been used for prevent and treatment of this neurogenerative dysfunction (2).

Centella asiatica urban (umbelliferae) is commonly used in traditional medicine for improvement of bruising and inflammation. The plant is claimed to possess anti-inflammatory (3), memory improvement (4) and anticancer activity (5). CA is also useful in venous hypertension and atheroscelerosis (6, 7). For antioxidant activity, CA has been reported to have anti-lipid peroxidative and free radical scavenging activities. CA extract is also has protective effect against adriamycin induced cardiomyopathy (8). Recently, Ramanathan et al. suggested that protective effect of CA extract on monosodium glutamate induced neurodegeneration attributed to is antioxidant properties (9). Therefore, the present work was performed to confirm the antioxidative and neuroprotective activities of CA extract by using different methods. We here also evaluated effect of different kinds of extracts that is chloroform, methanol and water extracts.

Material and Methods

Preparation of plants

Air-dried and powdered leaves of CA were extracted with chloroform, methanol and water. The supernatant was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The yields of the chloroform, methanol and water extracts were 4.12, 20.55 and 54.12 % respectively.

Cell cultures

Neuroblastoma NG108-15 cells were grown in DMEM containing 100 μM hypoxanthine, 1 μM aminopterin and 16 μM thymidine and 10 % fetal bovine serum. Cell cultures were maintained in a humidified incubator with 5% CO_2 – 95% air at 37 °C.

DPPH radical-scavenging activity

The free radical scavenging activities of the extracts were determined with 1,1-diphenyl-2-picryl-hydrazil (DPPH.). The radical scavenging activity of DPPH was expressed as IC_{50}. This value represents the concentration of a test compound required to inhibit 50% of the initial DPPH free radical.

Hydrogen peroxide–induced neurotoxicity and study of protection offered by extracts

NG108-15 cells ($2x10^3$cells/well) were seeded into 96-well plates and incubated at 37 °C for 48 hr. After plating, they were treated with various concentrations of the three extracts or trolox for 2 hr, and a stock solution of H_2O_2 solution was added. Neuronal survival was quantified using MTT. The resulting colored end product was solubilized in dimethyl sulfoxide (DMSO) and measured using a Microplate Reader (A Packard Bioscience Company, USA) at 550 nm.

Statistics

All data are expressed as mean ± SD. Pearson's correlation analysis (SPSS 7.5 for Windows, SPSS Inc.) was used to test for the significance of the relationship between the concentration and percentage inhibition at a $p \leq 0.05$.

Results

DPPH radical scavenging activity

Trolox exhibited DPPH radical-scavenging activity with IC_{50} values of 3 μM (R^2 = 0.7853). Vitamin C showed DPPH radical-scavenging activity with IC_{50} values of 5 μg/ml (R^2 = 0.9064). The water (IC_{50} 62 μg/ml, R^2 = 0.9436) showed comparative radical-scavenging effects with methanol (IC_{50} 84 μg/ml, R^2 = 0.9430) and more efficient than chloroform extract (IC_{50} 243 μg/ml, R^2 = 0.7917).

Cytotoxic effects of H_2O_2 on neuroblastoma NG108-15 cells

NG108-15 cells were treated with various concentrations of H_2O_2 (100-800 μM). Following 2h of incubation after H_2O_2 challenge, cell viability was determined. The results showed that H_2O_2 100-800 μM significantly reduced cell viability in a concentration-dependent manner (Fig. 1). For subsequent protection experiments, we used 150 μM as the concentration of H_2O_2.

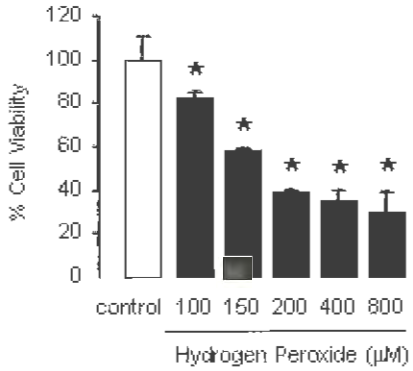

Figure 1 H_2O_2-induced cell damage in neuroblastoma NG108-15 cells
H_2O_2-induced reduction of viability of NG108-15 cells when treated with various concentrations of H_2O_2 (100-800 μM). After 2 h of incubation, cell viability was measured using the MTT method. Data are expressed as mean ± S.D. (n = 5). * $p \leq 0.05$ compared with the control group.

Cytotoxic effects of extracts on neuroblastoma NG108-15 cells

NG108-15 cells were treated with various concentrations of extracts (0.1-1000 μg/ml). Following 4h of incubation, cell viability was determined. The results showed that water and chloroform extracts exhibited cytotoxic effect at the concentration of 1000 μg/ml (Fig. 2 A,B) whereas methanol extract (0.1-1000 μg/ml) did not showed cytotoxic effect on cells (Fig. 2C).

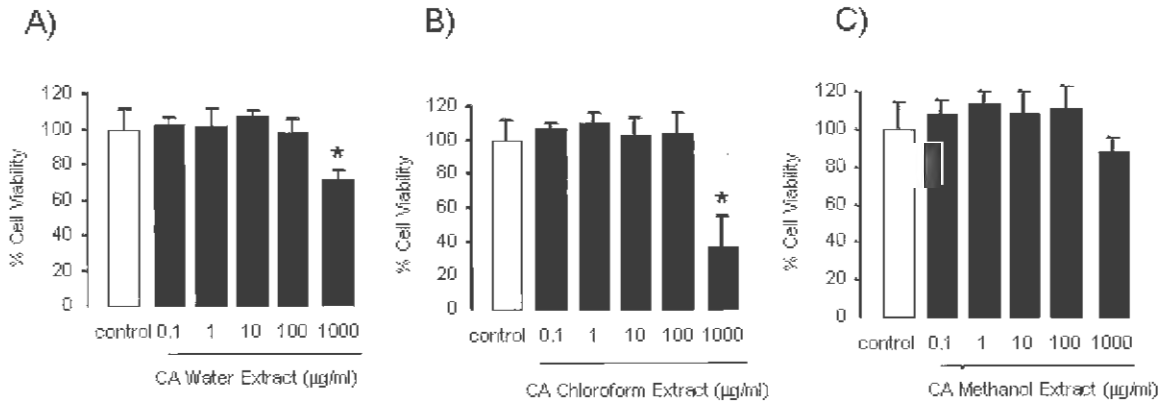

Figure 2 Cytotoxic effects of extracts on neuroblastoma NG108-15 cells
NG108-15 cells were treated with various concentrations (0.1-1000 μg/ml) of A) water B) chloroform and C) methanol extracts of *Centella asiatica*. After 4 h of incubation, cell viability was measured using the MTT method. Data are expressed as mean ± S.D. (n = 5). * $p \leq 0.05$ compared with the control group.

Effects of extracts on H_2O_2-induced neurotoxicity

When trolox, a reference standard, was added simultaneously with H_2O_2, it caused a concentration-dependent increase in cell viability from 1 to 100 μM (Fig. 3A). When the methanol extracts of CA (1-1000 μg/ml) were added together with H_2O_2, it significantly increased the viability of NG108-15 cells in a concentration dependent manner compared with treatment with H_2O_2 alone. The neuroprotective effects of methanol extracts of CA were observed in the concentration ranged of 10-100 μg/ml (Fig. 3D) whereas the water and chloroform extracts did not showed any neuroprotective effect Fig. 3B, C).

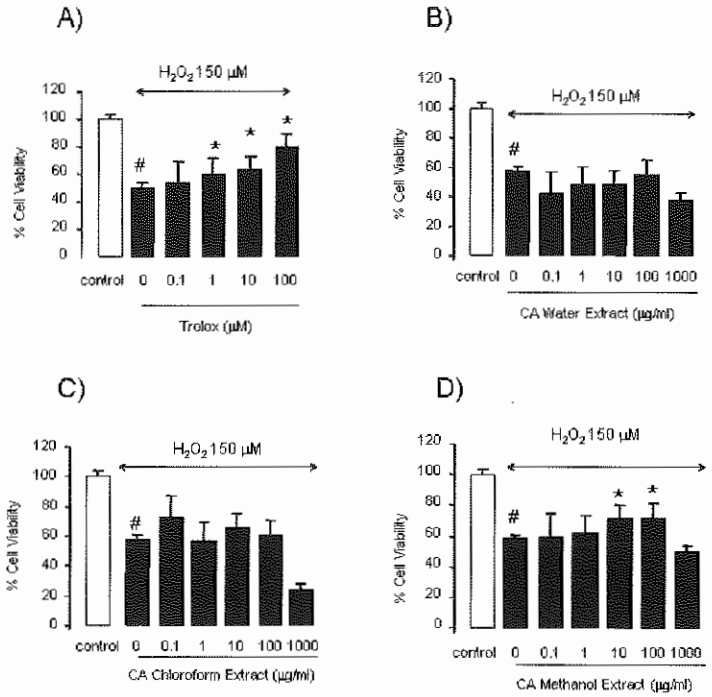

Figure 3 Effects of test compounds on H_2O_2-induced cell damage in NG108-15 Cells NG108-15 cells were treated with H_2O_2 (150 µM) together with various concentrations of test compounds: (A) reference drug, trolox, (B) water (C) chloroform and (D) Methanol extract of *Centella asiatica*. After 2 h of incubation, H_2O_2 (150 µM) was added, cell viability was measured using the MTT method. Data are expressed as mean ± S.D. (n = 5). # $p \leq 0.05$ compared with the control group. * $p \leq 0.05$ compared with the H_2O_2-treated control group

Discussion

Neurodegenerative diseases are characterized by the loss of neuronal cells in the brain. Reactive oxygen species (ROS) may be involved in the etiologies of these diseases. Antioxidant in plants or herbs may be useful in delaying or preventing of oxidation damages.

Our results demonstrate that all of extracts could potently reduce the stable radical DPPH. This indicated hydrogen donating ability. Among all extracts, only methanol extracts of CA showed neuroprotective against H_2O_2-induced toxicity in neuroblastoma NG-108. The neuroprotective effect of methanol extract may be partly due to its radical scavenging effect and may results from the active constituents present in the CA extract. The active constituents are triterpenes namely asiatic acid, asiaticoside (10). Also, different parts of CA were found to contain high phenolic contents, which exhibit strong association with its antioxidative activities (11). Asiatic acid has been reported to possess hepatoprotection and protective effects against β-amyloid-induced and glutamate-induced neurotoxicity (12,13). Although the water extract exhibited highest free radical scavenging activities, it has no protective effect on neuroblastoma against oxidative damage. These results may due to its cytotoxic effect.

In conclusion, our results suggest that the methanol extracts of CA exert significant neuroprotective effects against hydrogen peroxide induced cell death in neuroblastoma cells. It may be partly attributed to their apparent antioxidant and free radical scavenging properties. Thus, methanolic extracts of CA contain active components that may be of value in preventing neurogenerative diseases in which free radical generation is implicated.

Acknowledgments

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P12 Effects of the Standard Extract of *Centella asiatica* (ECa 233) on Phase II Drug Metabolizing Enzymes in Rat Livers

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Abstract

Introduction: *Centella asiatica* (Linn.) Urban is widely used as food, beverage and traditional medicine. The standard extract of *C. asiatica* (ECa 233) has been studied for an indication of memory enhancer.

Objective: We investigated the effects of ECa 233 on the activity of hepatic phase II drug metabolizing enzymes using rats liver cytosols.

Materials and methods: Male Wistar albino rats were treated with either ECa 233 (10, 100 or 1,000 mg/kg/day, p.o.) or vehicle control for 90 days. Liver cytosols were prepared and used for analysis the activities of sulfotransferase (SULT), glutathione *S*-transferase (GST) and NAD(P)H Quinoneoxidoreductase (NQOR).

Results: The result demonstrated that both doses of ECa233 caused a decrease of SULT activity, whereas the activities of GST and NQOR were not changed.

Discussion and Conclusion: The inhibitory effect of ECa 233 on SULT activity suggested the possibility of drug interaction on medicines that are metabolized by this enzyme. No effects of ECa 233 on GST and NQOR activities suggested no advantage effect of this extract regarding the detoxification of xenobiotics via these enzymes.

Key words: *Centella asiatica*, Phase II Drug Metabolizing Enzymes

Introduction

Centella asiatica (Linn.) Urban is a traditional plant of which stems and leaves are used for preparing beverage, consumed as food and taken as traditional medicine. The major constituents are triterpenoids, mainly asiatic acid, asiaticoside, madecassic acid and madecassoside (1).

The standard extract of *C. asiatica* (ECa 233) has been studied preclinically for an indication of memory enhancer by Tantisira M. et al. at the Faculty of Pharmaceutical Sciences, Chulalongkorn University. During research and development process of this extract for this indication, a study regarding effects of this extract on hepatic drug metabolizing enzymes either phase I and phase II metabolism are required. Modulation of this extract on hepatic phase I and phase II drug metabolizing enzymes would provide information regarding drug interaction and the possibility of the extract to increase/decrease risks of xenobiotic-induced toxicity/mutagenesis/carcinogenesis. Effect of ECa 233 on phase I enzymes (CYPs) has been studying. Thus, the aim of this study was to investigate effect of ECa 233 on hepatic phase II enzymes such as sulfotransferase (SULT), glutathione S-transferase (GST) and NAD(P)H quinoneoxidoreductase (NQOR) using rat livers cytosol.

Materials and Methods

Materials

These following chemicals were purchased from Sigma Chemical Co. Ltd., USA: adenosine 3'-phosphate 5'-phosphosufate (PAPS), bovine serum albumin, cupric sulfate, 1-chloro-2,4-dinitrobenzene (CDNB), 2,6-dichlorophenol-indophenol (DCPIP), dicumarol,

ethylenediaminetetraacetic acid (EDTA), Folin & Ciocalteu's phenol reagent, glutathione reduced from (GSH), magnesium chloride, nicotinamide adenine dinucleotide reduced from (NADH). Ethanol and potassium dihydrogen phosphate were purchased form Merck, Germany. 2-Naphthol was purchased from Aldrich, USA.

Animal treatment

Male Wistar albino rats (8 weeks old and weighing 250-300 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom, Thailand. Rats were housed at the Department of Medical Sciences, Ministry of Public Health, Thailand. They were maintained at 22-25 °C with 12-h light/dark cycle and allowed free access to standard diet and water throughout the study. The animals were allowed to acclimatize for seven days before the study. Rats (10 per group) were randomly assigned to the various treatment groups. Rats were orally gavaged with ECa 233 at doses of 10, 100 or 1,000 mg/kg/day or water for 90 days. At the end of the extract administration, rats were euthanized by diethyl ether inhalation. Livers were perfused with ice-cold saline and removed. Liver cytosols were prepared by a differential centrifugation method (2) with some modification and stored at -80 °C until enzymes assays. Protein concentrations of liver cytosols were determined by the method of Lowry et al (1951) (3).

Enzymes assay

Cytosolic SULT activity was determined using the spectrophotometric method described by Frame et al. (2000) (4) with some modification. 2-Naphthol was used as a selective substrate for the assay.

Cytosolic GST activity was determined spectrophotometrically at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as a selective substrate according to the procedure of Habig et al. (1980) (5) with some modification.

Cytosolic NQOR activity was determined by the method modifies from the method of Ernster (1990) (6) using 2, 6-dichlorophenol-indophenol (DCPIP) as a selective substrate.

Statistical analysis. Data were presented as means $\pm$ SEM. Statistical differences were determined by one-way analysis of variance, followed by Student-Newman-Keuls test. $P < 0.05$ was chosen as indicating significance.

Results

A significant decrease in the activity of SULT was observed in rats treated with ECa 233 at all doses used in this study (10, 100 or 1,000 mg/kg/day) as compared to the control group (Figure 1).

ECa 233 given at all dosage regimens used in this study did not affect the activities of NQOR (Figure 2) and GST (Figure 3).

Discussion and Conclusion

The inhibitory effect of ECa 233 on SULT activity suggested the possibility of drug interaction of this extract on medicines that are metabolizes by this enzyme. No effects of ECa 233 on GST and NQOR activities suggested no advantage effect of this extract regarding the detoxification of xenobiotics via these enzymes.

Acknowledgements

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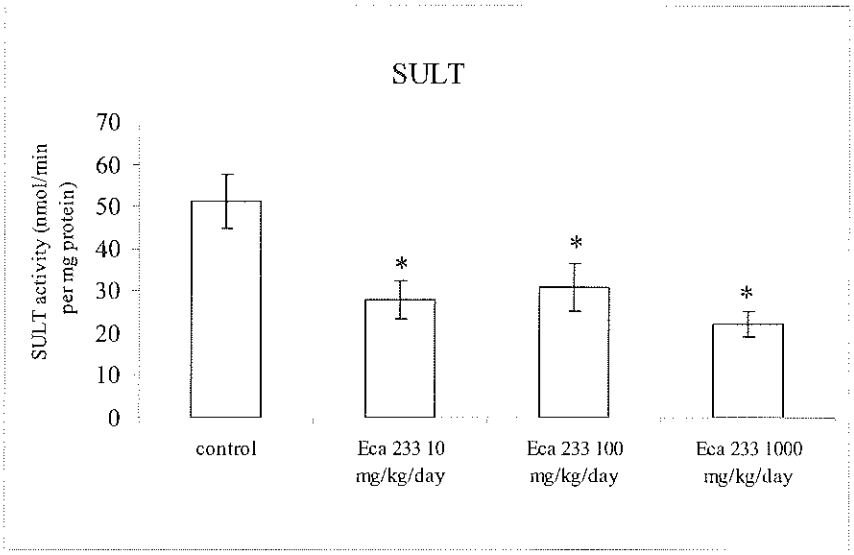

Figure 1 Effect of ECa 233 on hepatic cytosolic SULT activity. Data are presented as mean±SEM (n=10). * $P < 0.05$; ECa 233 treated group vs control group.

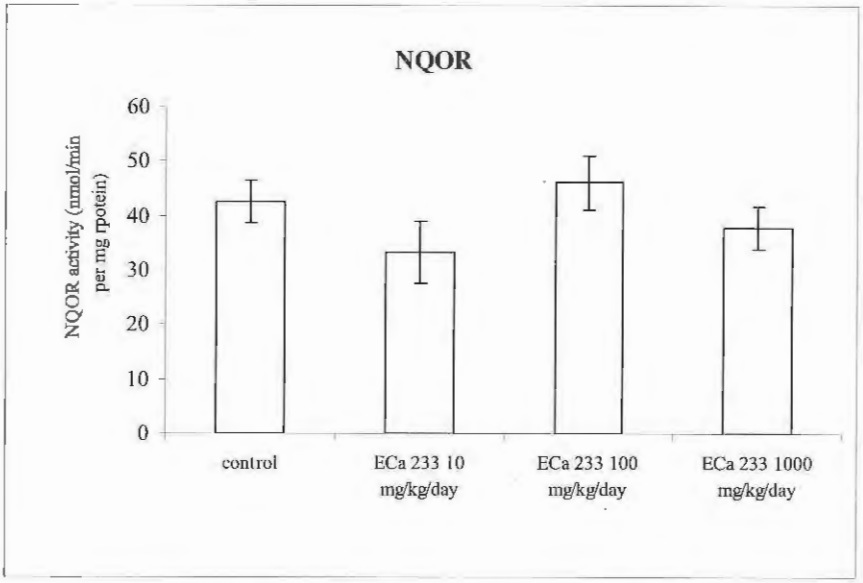

Figure 2 Effect of ECa 233 on hepatic cytosolic NQOR activity.

Data are presented as mean±SEM (n=10).

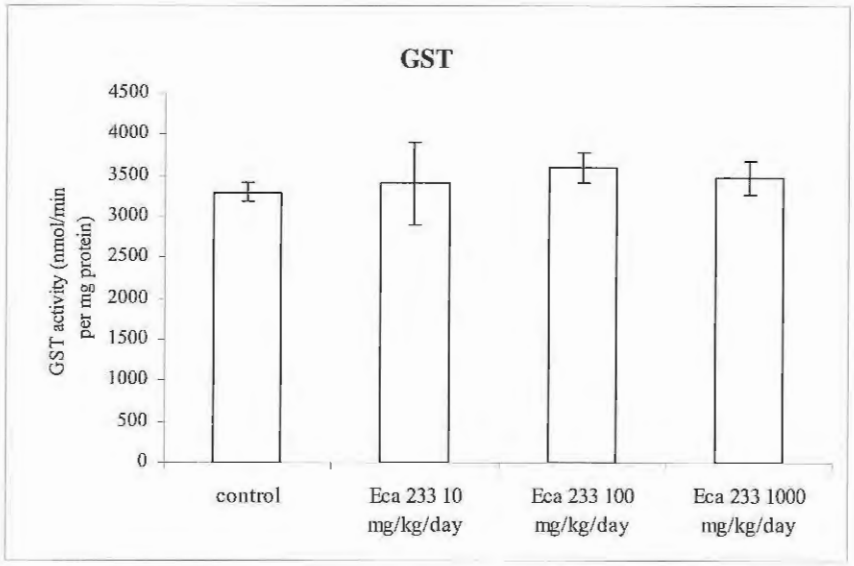

Figure 3 Effect of ECa 233 on hepatic cytosolic GST activity.
Data are presented as mean±SEM (n=10).

P13 Protective Role of Green Tea Extract on Acute Hepatitis in rat.

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Abstract

This study was planned to determine the protective role of green tea extract, if any, in attenuating the acute hepatitis induced by carbon tetrachloride (CCl_4). To assess the hepatitis, we determine hepatic marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum, including lipid peroxidation and histological changes in liver. Male Wistar rats were pretreated with green tea extract at dose of 1000, 2000 and 3000 mg/kg by single gavage for 24 hours before they given CCl_4 at dose of 1 ml/kg by intraperitoneally. All rats were killed 24 hours post-exposure. The results showed that activities of serum AST and ALT in green tea extract group were not different from control group. Lipid peroxidation induced by CCl_4 could be reduced significantly by green tea extract at dose of 2000 mg/kg ($p<0.05$). In addition, the vacuolation and necrosis of liver observed in this dose of green tea extract was less than other groups of treatment. This study concluded that green tea extract at dose of 2000 mg/kg could attenuate the acute hepatitis induced by CCl_4 that shown by lipid peroxidation and histological changes. This result indicated that green tea extract might have some protective role over the hepatitis by free radical scavengers.

Keywords: green tea, hepatitis, lipid peroxidation, ALT, AST

P14 Reversing β-Lactam Antibiotic Resistant Bacteria with Flavonoids

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Abstract

Resistance to β-lactam antibiotics is a global problem. Today over 90% of *Staphylococcus aureus* strains are β-lactamase positive. In addition, strains of methicillin-resistant *S. aureus* (MRSA) and ceftazidime-resistant *Enterobacter cloacae* (CREnC) are usually multiply resistant to many antibiotics and pose life-threatening risks to the hospitalised patients and their care givers. The search for new antibacterial agents and compounds that can reverse the resistance to β-lactam antibiotics are research objectives of far reaching importance and urgently needed. In this study, we have examined the antibacterial action of naturally occurring flavonoids. When combined ampicillin, cloxacillin and ceftazidime with baicalein 5 μg/ml, minimum inhibitory concentrations (MICs) of these drugs against clinical isolates of MRSA were reduced from 100, >1,000 and 50 μg/ml to 5, 5 and 5 μg/ml respectively. Furthermore, clinical isolates of CREnC with MICs of ceftazidime > 1,000 μg/ml had their resistance to these drugs reversed by apigenin 5 μg/ml or luteolin 5 μg/ml to MICs of ceftazidime 5 μg/ml. Viable counts showed that the killing of MRSA cells by 10 μg/ml ampicillin or cloxacillin was potentiated by 10 μg/ml baicalein. Ceftazidime 10 μg/ml in combination with 10 μg/ml of baicalein or galangin or quercetin also reduced the CFU/ml of MRSA to low levels ($1x10^3$ CFU/ml) over 6 h. The killing curve of CREnC cells were also maintained at low level from 6 to 24 h by ceftazidime 10 μg/ml in combination with 10 μg/ml of luteolin or apigenin. Electronmicroscopy clearly showed that the combination of 10 μg/ml baicalein with 10 μg/ml of ampicillin or cloxacillin and 10 μg/ml ceftazidime with 10 μg/ml of baicalein or galangin or quercetin caused damage to the ultrastructure of MRSA. Ceftazidime 10 μg/ml in combination with 10 μg/ml of luteolin or apigenin also caused marked morphological damage for CREnC. Enzymes assays indicated that galangin, baicalein, and quercetin had inhibitory activity against β-lactamase I from *Bacillus cereus*. Apigenin showed marked inhibitory activity against penicillinase type IV from *Enterobacter cloacae*.

From the study, it was concluded that baicalein, galangin and quercetin have the potential to reverse bacterial resistance to β-lactam antibiotics against MRSA. Luteolin and apigenin have synergistic effect with ceftazidime against CREnC. In view of their limited toxicity, These tested flavonoids offer for the development of a valuable adjunct to β-lactam treatments against otherwise resistant strains of currently almost untreatable microorganisms.
Keywords: β-lactam antibiotic, Resistant bacteria, Flavonoids

Introduction

Bacterial resistance to β-lactam antibiotics is a global problem. Today over 90% of *Staphylococcus aureus* (*S. aureus*) strains are β-lactamase positive (1). Strains of β-lactam-resistant *S. aureus* including methicillin-resistant *S. aureus* (MRSA) and strains of Ceftazidime-resistant *Enterobacter cloacae* (CREnC) now pose serious problem to

hospitalized patients, and their care providers (2). Antibiotics available for the treatment of MRSA or CREnC infection are fairly toxic and their use is frequently associated with unwanted side-effects (3). Novel antibiotics and/or new approaches that can reverse the resistance to well tried agents which have lost their original effectiveness are research objectives of far reaching importance (4). In this study, we have investigated the in-vitro activity of naturally occurring flavonoids, a major constituent in edible plants and / or traditional herbal remedies (5,6) against β-lactam-resistant *S. aureus* (MRSA) and CREnC when used alone and in combination with β-lactam antibiotics.

Materials and Methods

Flavonoids, β- lactam antibiotics and bacterial strains sources

All tested flavonoids (galangin, baicalein, apigenin, luteolin and quercetin) were obtained from Indofine Chemical company, New Jersy, USA. Ampicillin, cloxacillin and ceftazidime were obtained from Sigma. Three clinical isolates of methicillin-resistant *S. aureus* DMST 20651 (MRSA), Ceftazidime-resistant *Enterobacter cloacae* DMST 21394 (CREnC) and ampicillin sensitive *Enterobacter cloacae* DMST 19022 (ASEnC) were obtained from clinical microbiology department, Maharat Nakhonratchasima hospital, Nakhonratchasima and Department of Medical Sciences, Ministry of Public Health.

Bacterial suspension standard curve.

To select bacterial suspensions with a known viable count the following steps were followed Lui *et al.* (7).

Minimum inhibitory concentration (MIC) determinations

MIC derterminations of three clinical isolates of MRSA, CREnC and ampicillin sensitive *Enterobacter cloacae* (ASEnC) were followed Lui *et al.* (7) and NCCLS (8).

Checkerboard determinations

Checkerboard determinations in antimicrobial combinations were performed as previously described (9) with slight modification (10).

Killing curve determinations

Viable counts for the determination of killing-curves were performed as previously described by Richards and Xing (11).

Enzyme assays

The β-lactamases of *Bacillus cereus* (*B. cereus*) and *Enterobacter cloacae* (*E. cloacae*) were obtain from Sigma (Poole, England). Enzymes activities were followed Eumkeb and Richards (12).

Electronmicroscopy

Selected flavonoids that dramatically decreased the MICs of selected β-lactam antibiotics when used in combination will be used to examine electronmicroscopy. Therefore, the following flavonoids were chosen for electronmicroscopy study when used singly and in combination. Subculture of MRSA and CREnC were prepared to examine by TEM following Richards *et al.* (13).

Results

MIC determinations

The MICs for the tested flavonoids and β- lactam antibiotics against three clinical isolates strains of MRSA, CREnC and ASEnC are shown in Table 1. All tested flavonoids (galangin, baicalein, apigenin, luteolin and quercetin) showed no activity against all isolates strains of MRSA, CREnC and ASEnC at MICs of >400 μg/ml. CREnC was resistant to all tested β- lactams (ampicillin, cloxacillin and ceftazidime) at MICs of > 1,000 μg/ml. MRSA was resistant to ceftazidime, ampicillin and cloxacillin at MICs of 50, 100 and > 1,000 μg/ml. ASEnC was also resistant to ceftazidime and cloxacillin, while showed sensitive to ampicillin.

Checkerboard determinations

The isobolograms obtained from plotting of checkerboard MIC determinations showed the synergistic activity for all combinations of baicalein and all tested β- lactams against clinical isolates of MRSA. The MICs of ampicillin, cloxacillin and ceftazidime were reduced from 100, >1,000 and 50 μg/ml to 5, 5 and 5 μg/ml when combined with baicalein 5 μg/ml against this strain. Apigenin or luteolin 5 μg/ml reduced the MICs of both ampicillin and ceftazidime at > 1,000 μg/ml to 30 and 5 μg/ml respectively against this strain.

Killing curve determinations (Viable counts)

Sample killing curves resulting from selected β-lactam alone and in combination with selected flavonoids against MRSA and CREnC are presented in Fig.1 to 3. The control of both MRSA and CREnC showed no reduction in the counts of CFU from control inoculum.

Fig.1 shows that ampicillin, cloxacillin and baicalein at 50 μg/ml alone had little effect on the bacterial growth rate compared with the control. Baicalein at 10 μg/ml plus either ampicillin at 10 μg/ml or cloxacillin at 10 μg/ml reduced the viable counts by $5x10^2$ CFU/ml for MRSA in 6 h and to below the lowest detectable limit (10^3 CFU/ml) in 24 h.

Fig.2 shows that viable counts for MRSA were slight reduced by ceftazidime at 30 μg/ml, quecertin (50 μg/ml), galangin (50 μg/ml) or baicalein (50 μg/ml) alone when compared with the level of the untreated control culture between 6 to 24 h period. Ceftazidime (10 μg/ml) in combination with baicalein (10 μg/ml) or galangin (10 μg/ml) or quecertin (20 μg/ml) reduced the CFU/ml by $5x10^2$ over 6 h in all combinations. The reduced counts did not recover in 24 h.

Similar decreases in resistance of microorganisms to antibiotics were obtained with combination of ceftazidime with luteolin or apigenin against CREnC, both luteolin (10 μg/ml) and apigenin (10 μg/ml) in combination with ceftazidime at 10 μg/ml reduced the CFU/ml count by $5x10^2$ over 6 h. The reduced counts did not recover in 24 h. While ceftazidime (30 μg/ml), apigenin (50 μg/ml) and luteolin (50 μg/ml) alone had little effect on the bacterial growth rate compared with the control (Fig.3).

Electronmicroscopy

Fig. 4a shows the appearance of normal log phase cells of clinical isolates of MRSA. The cell wall and the cytoplasmic membrane can be distinguished. The electron dense ribosomes can be seen in great number in cytoplasm. The micrographs of this strain after exposure to cloxacillin 20 μg/ml show some of the bacteria have a little larger than those of control cells. (Fig. 4b) The micrograph of MRSA treated with ampicillin 20 μg/ml show that some of the bacteria have slightly larger gap between the cytoplasmic membrane and cell wall (Fig 4c). Electronmicroscopy clearly showed that the combination of 10 μg/ml baicalein with 10 μg/ml of ampicillin or cloxacillin caused electron-transparent areas of MRSA devoid of ribosomes (holes) clearly visible within the cytoplasm. A lot of cells lost some organelles from cytoplasm such as ribosomes chromosomes etc. Some of these showed morphological damages such as cell wall and cell shape distortion. Broken of some cells are also observed.

Moreover, the combination between 10 μg/ml ceftazidime with 10 μg/ml of baicalein or galangin or quecertin caused damage to the ultrastructure of MRSA such as cell wall damage, electron-transparent area devoid of ribosomes and lost some organelles from cytoplasm (Fig. 4d, 4e, 4f, 4g, 4h)

Fig.5 a shows the appearance of normal log phase cells of clinical isolates of CREnC. The outer membrane and the cytoplasmic membrane can be distinguished. The electron dense ribosomes can be seen in numerous number in cytoplasm. The micrographs of CREnC after exposute to ceftazidime 20μg/ml show some of these bacterial cells exhibited larger gap between outer membrane and cytoplasmic membrane (Fig.5b)

The resulted showed that ceftazidime 10 μg/ml in combination with 10 μg/ml of luteolin or apigenin also caused marked morphological damage for CREnC. A lot of these bacterial cells exhibited morphological damage of cell wall and cell shape and electron-transparant area in cytoplasm due to losing most of organelles. Several bacterial cells showed broken cell and distortion of cell wall. The micrographs of CREnC after exposure to ceftazidime 10 μg/ml plus apigenin 10 μg/ml showed obvious detachment of cell wall and plasma membrane. Distortion of cell wall in several bacterial cells are also observed (Fig.5c, 5d).

Enzyme assays

The ability of flavonoids to inhibit the *in vitro* activity of β-lactamases varied considerably. Fig.6 indicates that galangin has an inhibitory activity against β-lactamaseI from *B. cereus*. Galangin had some activity and tectochrysin and 6-chloro-7-methylflavone showed greater activity. Against penicillinase type IV from *E. cloacae*, apigenin showed marked inhibitory activity but none of other flavonoids tested showed appreciable activity. These results indicate that in addition to the direct effect on cell structure and cell division, the resistance reversing activity of flavonoids against bacteria might also include inhibition of β-lactamase activity.

Discussion and Conclusion

The results of checkerboard and viable counts of MRSA are in substantial agreement with those of Lui (7) that baicalin had the potential to restore the effectiveness of β-lactam antibiotics against MRSA. The results seem consistent with Sato *et al.* (14) that 6,7 dihydroxyflavone synergistically elevates the susceptibility of MRSA to β-lactam antibiotics from 8- to 32,000-fold. Moreover, flavone found to show diverse synergistic effects on the susceptibility of MRSA to β-lactam antibiotics (15). The TEM results of MRSA seem consistent with Eumkeb and Richards (12) that the combination of β-lactam with galangin caused damage to the ultrastructural of MRSA cells. These results indicated that flavonoids not only have an activity of their own against β-lactam-resistant staphylococci but also have the ability to reverse the resistance of such bacterial strains to the activity of the primary antibiotics. This may involve two mechanisms of action by the flavonoids. The first is on the integrity of the cell wall and on septum formation prior to cell division. This implies an effect on protein synthesis including an effect on penicillin-binding proteins. The second mechanism of β-lactam activity is via inhibition of the activity of certain β-lactamase enzymes. The first action could also include an effect on the production and/or release of β-lactamase enzymes within and from the cell walls (16). In the last two decades, β-lactamase inhibitors like clavulanic acid have played an important role in fighting β-lactam-resistant bacteria. These inhibitors work as suicide compounds to react with the enzymes since they share the same key structure with β-lactam antibiotics (17). Recent studies demonstrated that clavulanate caused a considerable induction of β-lactamase expression and an increase of clavulanate concentration was followed by an elevation in β-lactamase production (18,19). This indicates

that the presently available β-lactamase inhibitors can also lose their activity by the same mechanism as the β-lactam antibiotics. Our research provides an unique example that flavonoids without a β-lactam structure can reverse bacterial resistance to β-lactams via multiple mechanisms. Because of this structural dissimilarity these compounds are unlikely to induce β-lactamase production. It should also be remembered that conventional β-lactamase inhibitors, unlike flavonoids, cannot reverse the resistance of MRSA, which is one of the most dangerous bacterial pathogens.

From the study, it was concluded that baicalein, galangin and quecertin have the potential to reverse bacterial resistance to β-lactam antibiotics against MRSA. Luteolin and apigenin have synergistic effect with ceftazidime against CREnC. In view of their limited toxicity, These tested flavonoids offer for the development of a valuable adjunct to β-lactam treatments against otherwise resistant strains of currently almost untreatable microorganisms.

Acknowledgements

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Table 1 MICs (μg/ml) for tested flavonoids and β- lactams used againsts clinical isolates strains of MRSA, CREnC and ASEnC

	ampicillin	ceftazidime	cloxacillin	galangin	baicalein	apigenin	luteolin	quercetin
MRSA	100	50	>1000	>400	>400	>400	>400	>400
CREnC	>1000	>1000	>1000	>400	>400	>400	>400	>400
ASEnC	6	10	80	>400	>400	>400	>400	>400

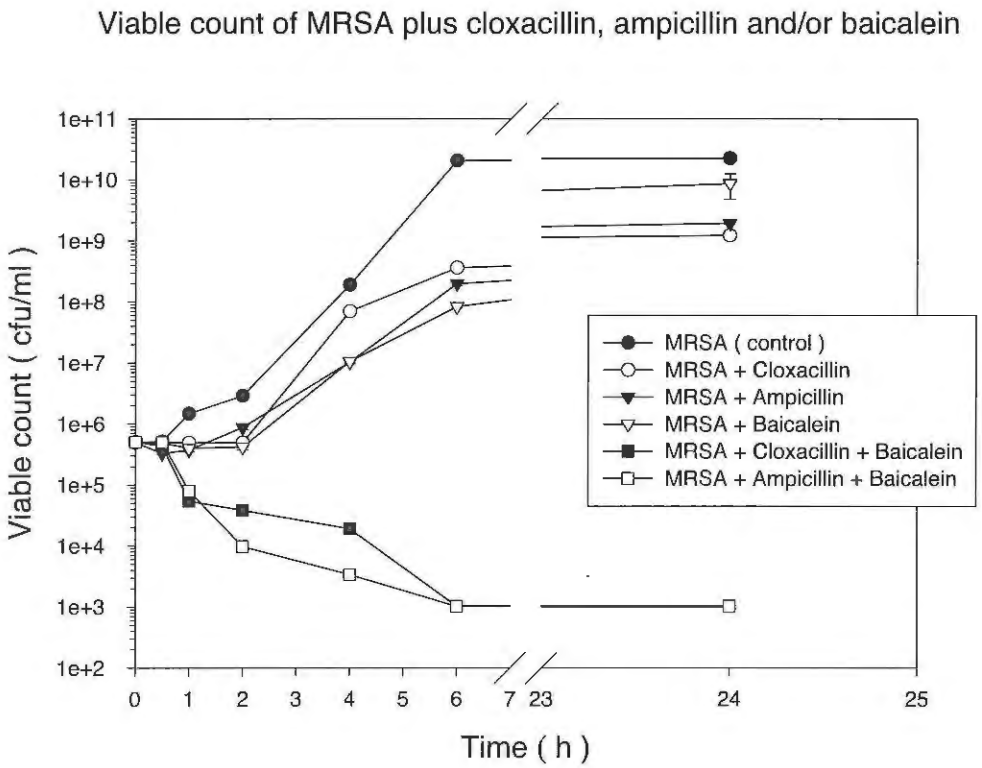

Figure 1 The effect of cloxacillin or ampicillin combined with baicalein on the viable counts of MRSA. ●, control (bacterial culture with corresponding solvent);○, cloxacillin 50 μg/ml ; τ, ampicillin 50 μg/ml ; ∇, baicalein 50 μg/ml ; ν , cloxacillin 10 μg/ml plus baicalein 10 μg/ml ; □ , ampicillin 10 μg/ml plus baicalein 10 μg/ml ;the values plotted are the means of 4 observations, and the vertical bars indicate the standard errors of the means.

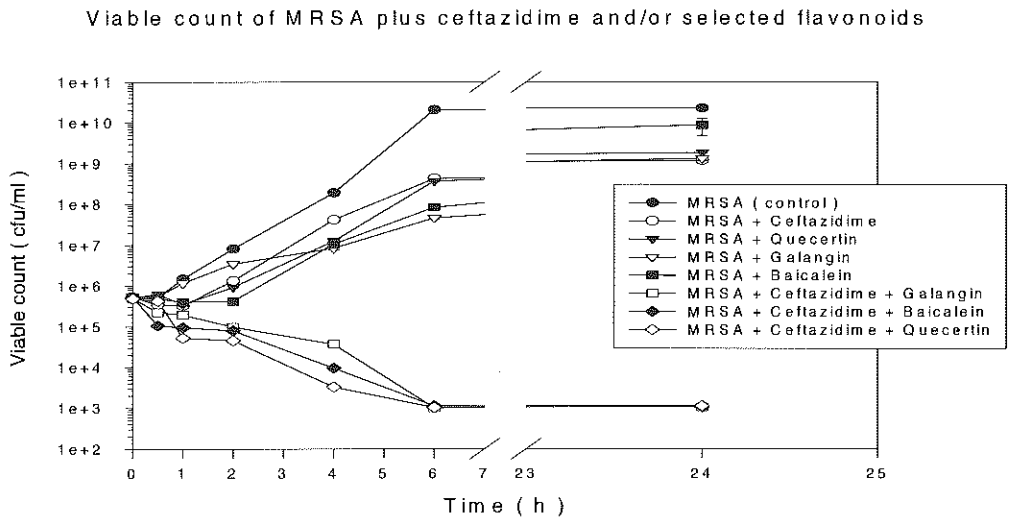

Figure 2 The effect of ceftazidime combined with selected flavonoids on the viable counts of MRSA. ●, control (bacterial culture with corresponding solvent); ○, ceftazidime 30 μg/ml ; τ, quecertin 50 μg/ml ; ∇, galangin 50 μg/ml ; ν , baicalein 50 μg/ml ; □ , ceftazidime 10 μg/ml plus galangin 10 μg/ml ; υ, ceftazidime 10 μg/ml plus baicalein 10 μg/ml ; ◇ ceftazidime 10 μg/ml plus quecertin 10 μg/ml ; the values plotted are the means of 4 observations, and the vertical bars indicate the standard errors of the means.

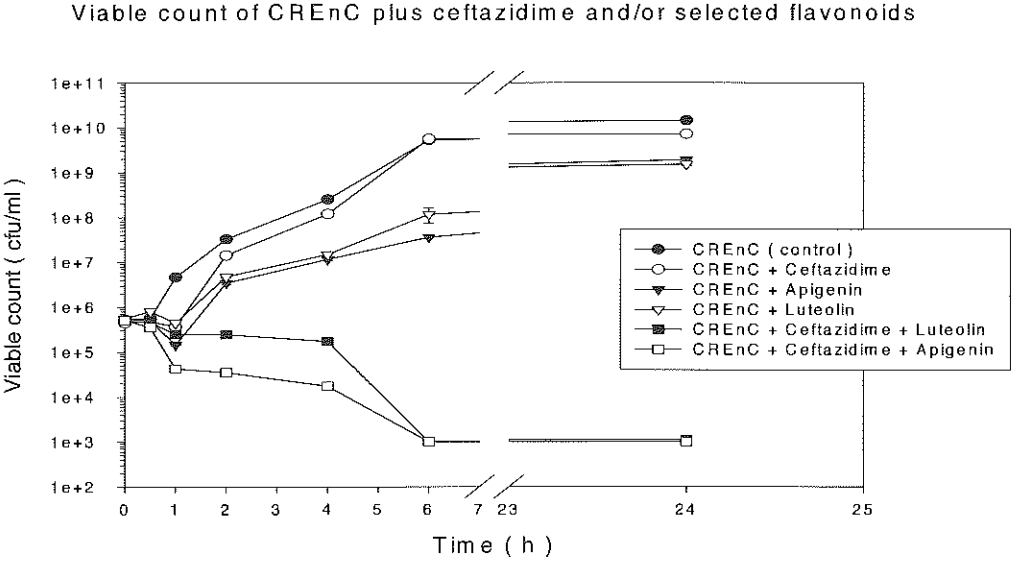

Figure 3 The effect of ceftazidime combined with selected flavonoids on the viable counts of CREnC. ●, control (bacterial culture with corresponding solvent); ○, ceftazidime 30 μg/ml ; τ, apigenin 50 μg/ml ; ∇, luteolin 50 μg/ml ; ν , ceftazidime 10 μg/ml plus luteolin 10 μg/ml ; □ , ceftazidime 10 μg/ml plus apigenin 10 μg/ml ; the values plotted are the means of 4 observations, and the vertical bars indicate the standard errors of the means.

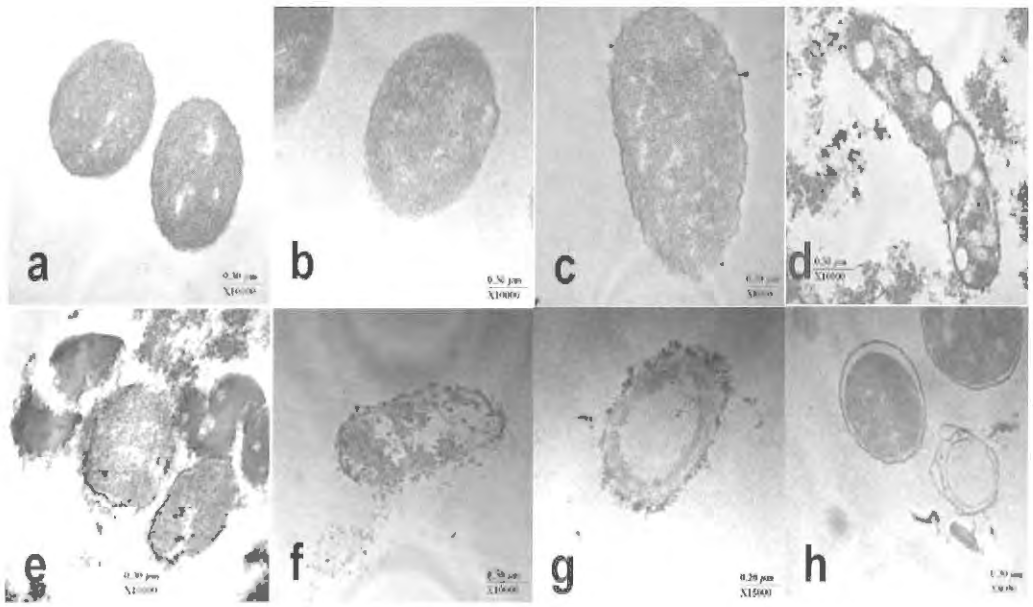

Figure 4 Ultrathin sections of log phase *S. aureus* DMST 20651 (MRSA) grown in Mueller-Hinton broth containing: **a**, drug-free (control); **b**, 20 μg/ml cloxacillin; **c**, 20 μg/ml ampicillin; **d**, 10 μg/ml cloxacillin plus 10 μg/ml baicalein; **e**, 10 μg/ml ampicillin plus 10 μg/ml baicalein; **f**, 10 μg/ml ceftazidime plus 10 μg/ml galangin; **g**, 10 μg/ml ceftazidime plus 10 μg/ml baicalein; **h**, 10 μg/ml ceftazidime plus 10 μg/ml quercetin.

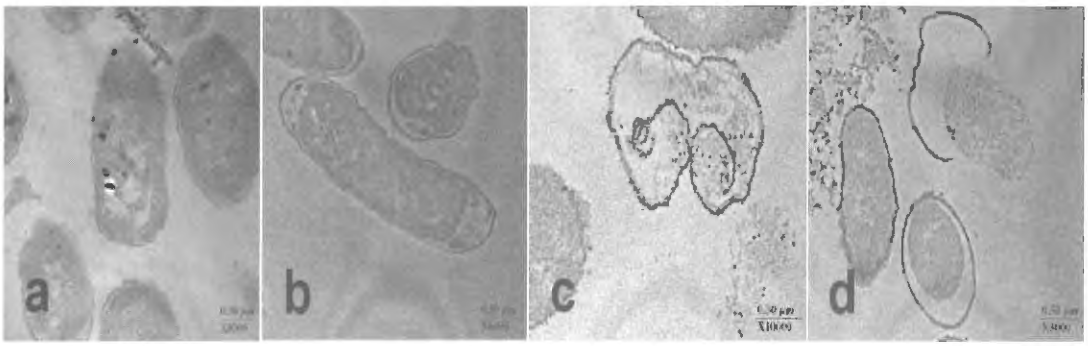

Figure 5 Ultrathin sections of log phase *E. cloacae* DMST 21394 (CREnC) grown in Iso-sensitest broth containing: **a**, drug-free (control); **b**, 20 μg/ml ceftazidime; **c**, 10 μg/ml ceftazidime plus 10 μg/ml luteolin; **d**, 10 μg/ml ceftazidime plus 10 μg/ml apigenin.

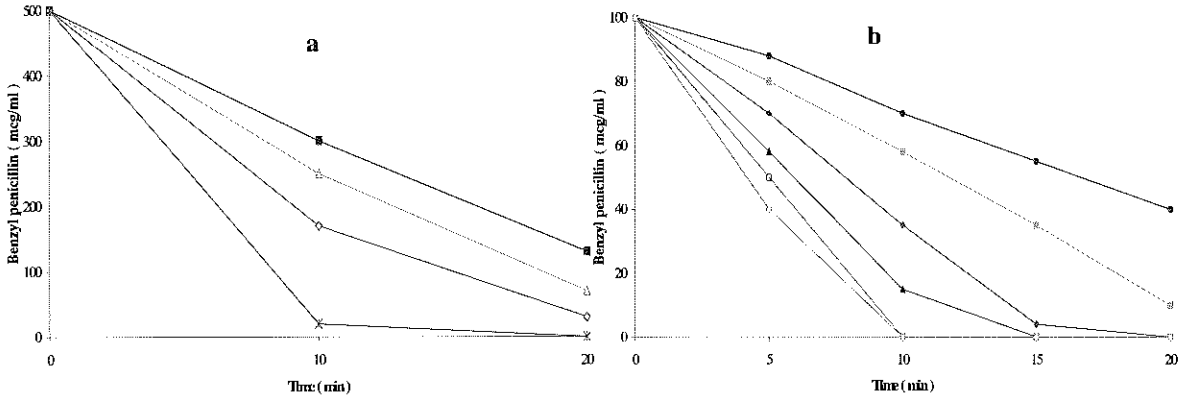

Figure 6 The inhibitory activity of flavonoids against β-lactamase in hydrolyzing benzylpenicillin. **a**. β-lactamase used from *B. cereus*; symbol represents flavonoids (200 μg/ml); ∗, control (without flavonoids); ◊, galangin; Δ, 6-chloro-7-methylflavone; ■, tectochrysin. **b**. β-lactamase used from *E. cloacea*; symbol represent concentrations (μg/ml) of apigenin; o, control (without apigenin); □, 20; σ, 40; υ, 60; ■, 80; •, 100.

P15 Characterization and Biological Activities of King Cobra (*Ophiophagus hannah*) venom

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Abstract

This study aimed at determining the quality specifications and biological effects of the crude King Cobra (*Ophiophagus hannah*) venom (KCV) obtained from three sources in Thailand. Physicochemical properties, including appearance; protein content/pattern; various enzyme activities were studied. Protein pattern of lyophilized KCV was determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and 2-dimension electrophoresis. Biological effects, including hemolysis, neurotoxicity, and coagulation were studied by the method described by Theakston and Reid. Cytotoxicity was tested in various cancer cell-lines, including BT 474 (breast cancer cell); Chago (lung cancer cell); Hep-G2 (hepatoma); KATO-III (stomach cancer); and SW 620 (intestine cancer). LD50 of the crude KCV was determined. The results showed that crude KCV was devoid of coagulation effect. KCV from different sources showed quantitatively different in hemolytic and neurotoxic effects. The neurotoxic effect was related to the LD50 of the crude KCV. Crude KCV showed IC50 (inhibitory concentration 50) on the growth of cancer cell lines comparable to doxorubicine, an anticancer agent. In conclusion, KCV from various sources have different potency of neurotoxicity. Fractionation of KCV to obtain the active compounds are advisable in order to develop new therapeutic agents, eg. neuromuscular blocking and anticancer agents.

Keywords: King Cobra venom, *Ophiophagus hannah*, protein pattern, biological Activity

P16 The Study of Radiochemical Stability of 2-deoxy-2-[F-18]-fluoro-D-glucose (^{18}F-FDG) Radiopharmaceutical

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Abstract

The ^{18}F-FDG radiopharmaceutical has become widely used in nuclear medicine for cancerous diagnostic studies using Positron Emission Tomography (PET) body technique. However, the ^{18}F-FDG solution rapidly loses in radiochemical purity due to both radiolysis and the half-life of the radioisotope. Thus the compound fails to meet the specifications dictated by European Pharmacopeia (EP) and United States Pharmacopeia (USP) and limiting the period in which the compound can be used. In this study we are investigate the effects of radioactive concentration and the ethanolic free radical scavenger on the radiochemical purity of ^{18}F-FDG preparations using the radio-TLC technique. Fifteen batches of ^{18}F-FDG were collected from routine production with radioactive concentrations in the range of 0.261-1.411 GBq/ml (0.979±0.425 GBq/ml) and the radiochemical purity at the end of synthesis (EOS) was 97.813±0.337%. Our results show that there is no radiolytic decomposition of ^{18}F-FDG and the radiochemical purity of these compounds maintains at more than 90% over 12 hrs from the EOS. However, the ^{18}F-FDG will exhibit radiolytic instability if prepared in relatively high concentration (>3.7 GBq/ml). In the presence of ethanol of at least 0.01% in the final product, the radiochemical purity of ^{18}F-FDG preparations was more than 95%. Thus ethanol seems to be a natural choice of stabilizer for the production of ^{18}F-FDG.

Keywords: ^{18}F-FDG, Radiochemical purity and Radiolysis

Introduction

Professor Dr. HRH Princess Chulabhorn Mahidol, the president of Chulabhorn Cancer Centre, in cooperation with three medical schools namely; Faculty of Medicine Siriraj Hospital Mahidol University; Faculty of Medicine Ramathibodi Hospital Mahidol University; Faculty of Medicine Chulalongkorn University and National Cancer Institute, has established the ***National Cyclotron and PET Centre*** in the year 2006 to provide a cancerous diagnostic including the researches, a global exchange of knowledge and know-how, as well as nuclear medicine services for cancerous-disease patients, brain and heart diseases.

In the recent years, the ^{18}F isotope-labeled glucose 2-deoxy-2-[F-18]-fluoro-D-glucose (^{18}F-FDG) has become widely used in nuclear medicine for diagnostic studies using a Positron Emission Tomography (PET) body technique. The compound, aside from important uses in cardiology and neurology, has shown an ability to detect cancerous tissues undetectable by conventional means or to correct misdiagnosis of the disease. This is due to exploiting a fundamental change that occurs in cells when they become malignant; cancer cells lose their ability to efficiently convert glucose into energy. Consequently, they require much more glucose, up to 20 to 50 times more (1).

Currently, the preferred method of producing the ^{18}F isotope is by bombarding water enriched with the ^{18}O isotope using high energy protons from a cyclotron. The synthesis of ^{18}F-FDG involves a nucleophilic ^{18}F fluorination step which to formation of an acetylated

derivative of FDG and then a hydrolysis step during which protective acetyl groups are removed resulting in the final product (2). However, the radiochemical purity of the compound decreases drastically during standard steps and thus the compound fails to meet the specifications dictated by European Pharmacopeia (EP) and United States Pharmacopeia (USP). In addition, after synthesis, the ^{18}F-FDG rapidly loses in radiochemical purity due to both radiolysis and the half-life of the radioisotope, limiting the period in which the compound can be used.

Objectives

1. Investigate the effects of radioactive concentration on the radiochemical purity of ^{18}F-FDG solutions over the time period.
2. Investigate the effects of ethanolic free radical scavenger on the radiochemical purity of ^{18}F-FDG solutions over the time period.

Materials and Methods

The ^{18}F-FDG was produced using the ^{18}O (p, n) ^{18}F nuclear reaction. Labeling was completed using the GEMS TRACERlab MX_{FDG} synthesis module (2). The final product was formulated in 0.9% NaCl solution and was not doped with stabilizing material. All quality assurance testing met the standards of the United States Pharmacopeia monograph (3).

The radiochemical purity of ^{18}F-FDG preparations was determined by radio-Thin Layer Chromatography (radio-TLC) according to the monograph using 5x20 cm TLC silica plates supplied by Fisher or equivalent. A 95:5 mixture of acetonitrile and water was used as a mobile phase. The radioactivity distribution on the plate was measured by a Bioscan TLC scanner using Winscan® software program at the time of 0, 2, 4, 8 and 12 hrs after the end of synthesis.

Ethanol concentrations were determined with Gas Chromatograph (GC) analysis using an HP 6850 gas chromatograph equipped with 50 m capillary column, type DB WAX, and a standard HP Flame Ionization Detector (FID). Helium flow was 15 ml/min with split injection and a constant temperature of 50°C (Figure 1).

Results

Fifteen batches of ^{18}F-FDG were collected from the routine production with radioactive concentrations in the range of 0.261-1.411 GBq/ml (0.979±0.425 GBq/ml) (Table 1). The radiochemical purity of these compounds at the end of synthesis in the mean±SD was 97.817±0.337% (Table 1, Figure 2).

The radiolytic decomposition profiles of ^{18}F-FDG preparations are determined at the time of 0, 2, 4, 8 and 12 hrs after the end of synthesis. The data shows that there is no radiolytic decomposition of ^{18}F-FDG is observed and the radiochemical purity of these compounds maintains at more than 90% over 12 hrs from the EOS. These values at the time of 0, 2, 4, 8 and 12 hrs from the EOS in the mean±SD were 97.813±0.337%, 97.439±0.527%, 97.255±0.493%, 96.929±0.775% and 96.177±1.372%, respectively (Figure 3).

The effects of ethanol concentration on the radiolytic decomposition of ^{18}F-FDG preparations are illustrated. The results show that, in the presence of ethanol in the final product of at least 0.01%, the radiochemical purity of ^{18}F-FDG preparations was more than 95% (Figure 4).

Discussion

These results support the fact that ^{18}F-FDG will exhibit radiolytic instability if prepared in relatively high concentration (>3.7 GBq/ml) (4). It is caused mainly by oxidation by free radicals that are produced by the interaction of ionizing radiation from the ^{18}F isotope

with the water solvent and possibly air. These processes may be lead to the decomposition of ^{18}F-FDG, which can be quantified in terms of decreased radiochemical purity. The radiochemical purity is typically expressed as a percentage of activity in the form of ^{18}F-FDG relatively to the total radioactivity present in the sample (5). The relative low radioactive concentration can be sufficiently protective against radiation-induced the decomposition of ^{18}F-FDG preparations (4).

The important goal for ^{18}F-FDG manufacturers is an improving the stability of ^{18}F-FDG preparations and increasing the radiochemical purity at the time of administration. It is also important to control radiolysis during the ^{18}F-FDG production steps to increase radiochemical yield of the product. Therefore, several stabilizers were used to extend the expiry time of the product (5). Currently, ethanol has been widely used as stabilizer for the preparation of ^{18}F-FDG. Because during the process of ^{18}F-FDG production, ethanol is incorporate into the final product, having a concentration in a range of minimum effective stabilization up to a practical pharmacopeia limit (3, 4, 5).

Conclusion

The radiochemical purity is an important indicator to determine the stability of ^{18}F-FDG preparations over a period of 12 hrs or longer after the end of synthesis. The quality standard established by USP for ^{18}F-FDG is not less than 90% radiochemical purity. It is obviously desirable to retain as high radiochemical purity as possible for as long as possible to achieve the best PET image quality. Our data support the role of ethanol as a free radical scavenger. The minimum effective ethanol concentration is about 0.01% (v/v) per GBq/ml of ^{18}F activity concentration is enough to maintain the stability of ^{18}F-FDG preparations.

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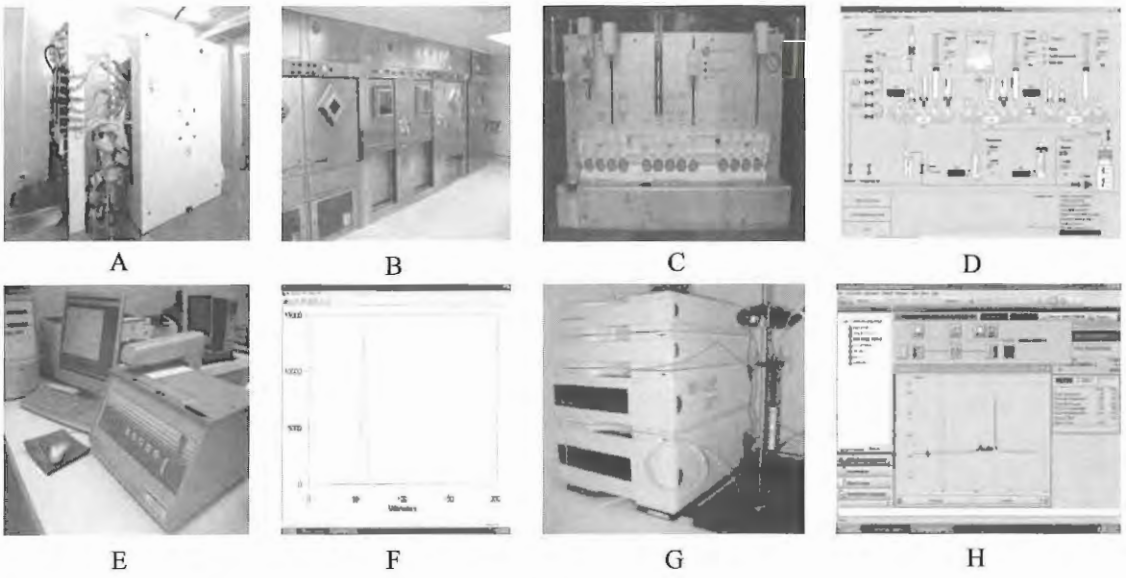

Figure 1 Schematic diagram of ^{18}F-FDG production and quality controls. A: Cyclotron, B: Radiochemistry room, C: TRACERlab MX_{FDG} synthesis module, D: ^{18}F-FDG production print screen, E: radio-TLC scanner, F: The radio-TLC report form, G: GC and H: GC report form.

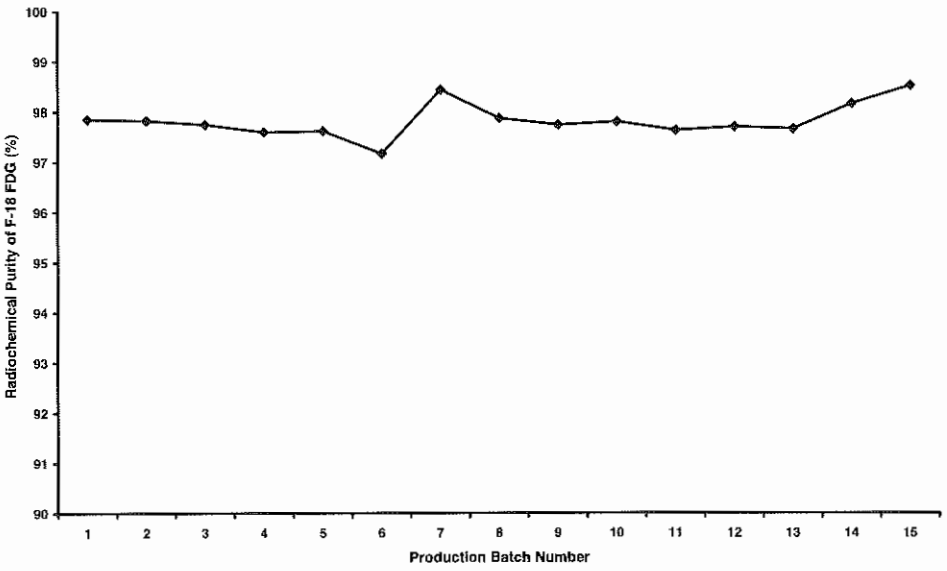

Figure 2 The percentages of radiochemical purity of ^{18}F-FDG preparations at the end of synthesis (t=0 hr) determined by radio-TLC.

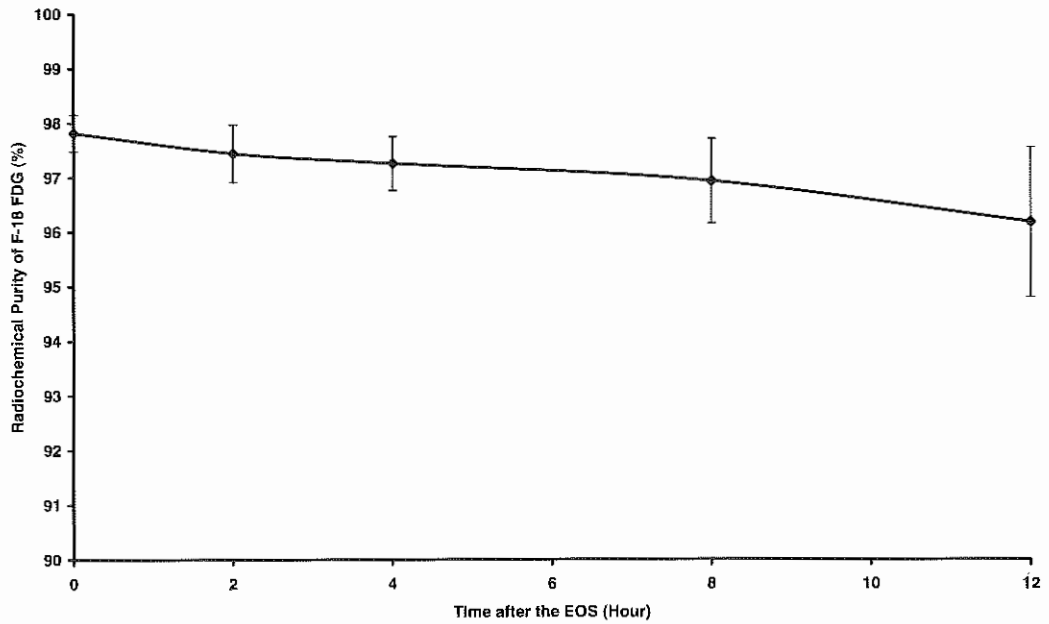

Figure 3 The radiolytic decomposition profiles of ^{18}F-FDG preparations over 12 hours after the end of synthesis.

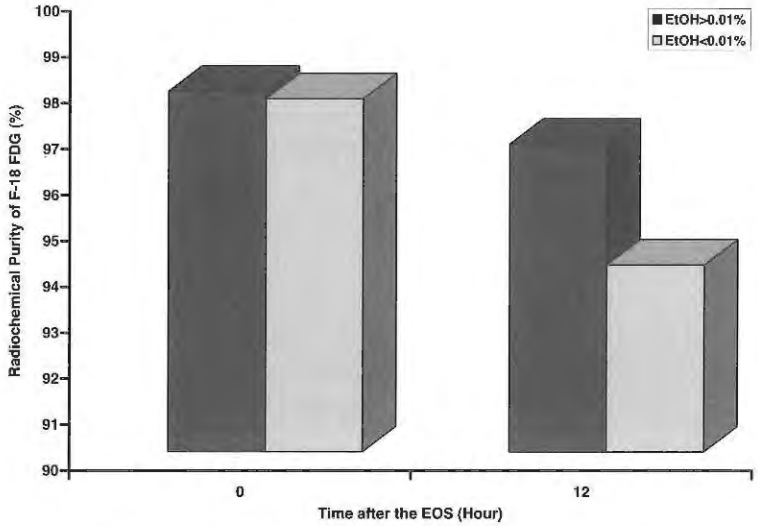

Figure 4 The effects of ethanol, comparison between low concentration of ethanol (<0.01%) and high concentration of ethanol (>0.01%) on the radiolytic decomposition of ^{18}F-FDG preparations.

Table 1 The results of radioactivity concentration, percentage of ethanol content and percentage of radiochemical purity of ^{18}F-FDG preparations at the end o

Production Batch Number	Radioactivity Concentration (GBq/ml)	Ethanol Content (%)	Radiochemical Purity (%) at the EOS
1	0.523	0.036	97.85
2	0.350	0.049	97.82
3	0.465	0.004	97.74
4	1.279	0.004	97.59
5	0.920	0.039	97.61
6	1.108	0.019	97.16
7	0.261	0.029	98.43
8	1.320	0.037	97.86
9	1.244	0.002	97.73
10	1.246	0.010	97.79
11	1.240	0.029	97.62
12	1.401	0.010	97.69
13	1.411	0.041	97.65
14	1.357	0.030	98.15
15	0.599	0.013	98.51

P17 A Novel Polymorphism in the Promoter Region of *UGT1A9* Gene in Thai Population

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Abstract

Introduction: The human uridine diphospho-glucuronosyltransferase, UGT1A9, catalyzes glucuronidations of various endobiotics and xenobiotics. Genetic polymorphisms in *UGT1A9* can influence detoxifying capacities and have considerable responses in the metabolisms of numerous drugs.

Objective: This study aimed to investigate the single nucleotide polymorphisms (SNPs) in the promoter region of *UGT1A9* gene in Thai population.

Materials and methods: Genomic deoxyribonucleic acid (DNA) samples from healthy unrelated volunteers were amplified by using polymerase chain reaction (PCR) technique. The PCR products were sequenced to identify the polymorphisms in the promoter region of *UGT1A9*.

Results: A novel SNP was identified in *UGT1A9* promoter region, heterozygous -689 (A>C).

Conclusion: This study showed a novel SNP in the promoter region of *UGT1A9* gene. However, further investigations of the possible influence of this polymorphism on enzyme activity should be carried out.

Key words: *UGT1A9*, polymorphism

Introduction

Uridine diphospho-glucuronosyltransferases (UGTs) represent one of the major classes of enzymes involved in phase II metabolism. UGTs are membrane-bound conjugating enzymes that catalyze the transfer of glucuronic acid moiety from uridine diphosphoglucuronic acid (UDPGA) to the functional group of a specific substrate to accelerate excretion from the body (1).

The UGT1A9 enzyme is encoded by the *UGT1A9* gene, which consists of 5 exons. Among the 5 exons, the first exon is unique, whereas exons 2-5 are common to all UGT1A subfamily members (2). To date, 27 variants of *UGT1A9* have been identified and published on the (http://www.pharmacogenomics.pha.ulaval.ca/webdav/site/pharmacogenomics/shared/Nomenclature/UGT1A/UGT1A9.htm). Some of these polymorphisms are known to affect glucuronidation activities (3-9). However, no such genetic polymorphisms of UGT1A9 in Thai population have been reported. Thus, the objective of the present study was to investigate the single nucleotide polymorphisms (SNPs) in the promoter region of *UGT1A9* gene in Thai population.

Methods

Genomic DNA samples were taken from healthy unrelated Thai volunteers who participated in the previous study (10). The promoters of the *UGT1A9* gene in Thai subjects were amplified by the polymerase chain reaction (PCR). For the genotyping, PCR products were sequenced to determine the polymorphisms.

Results

A total of 1,276 bps of the promoter *UGT1A9* gene was screened for genetic variations. Analysis of these sequences revealed a novel SNP at position -689 A>C, relative to the adenine of the predicted start codon (Accession number NG_002601). This SNP has not been reported elsewhere. The electrophoregrams of the mutation are shown in Figure 1.

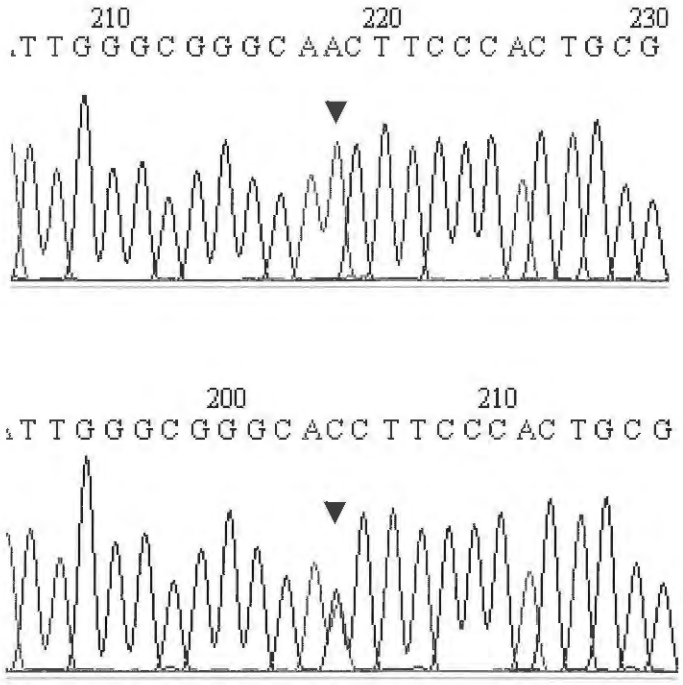

Figure 1 Electropherograms of *UGT1A9* gene sequences at nucleotide -677 to - 700 for wild-type (top) and heterozygous -689 A>C (bottom) individuals. Arrows indicate the variant nucleotide positions.

Discussion

The results from DNA sequencing of the 5′-regulatory region of the human *UGT1A9* gene showed a novel variation at nucleotide –689 (A>C). Thus, this is the first study to investigate genetic polymorphisms of the *UGT1A9* gene in Thai population. Study in a large sample size is required to elucidate the allele frequency of this SNP. However, tissue-specific expression of the *UGT1A9* genes appear to be regulated by promoter elements in the 5′-flanking region, further studies of the transcriptional activity influenced from this polymorphism should be carried out.

Conclusion

The novel SNP at nucleotide -689 (A>C) in the promoter region of *UGT1A9* gene in Thai population was found in this study.

Acknowledgement

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P18 Bioequivalence Study of a Generic Quetiapine in Healthy Thai Male Volunteers

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Abstract

Introduction: Quetiapine is an atypical antipsychotic used for the treatment of schizophrenia.
Objective: To study the bioequivalence of a generic quetiapine (Quantia 200®, manufactured by the Unison Laboratories Co., Ltd.) and the innovator product (Seroquel®).
Methods: The study was a randomized, two-way crossover design with a two-week washout period in 24 healthy Thai male volunteers. After a single 200-mg oral dosing, serial blood samples were collected at appropriate interval up to 48 h. Plasma quetiapine concentrations were determined by high performance liquid chromatography (HPLC). Pharmacokinetic parameters were estimated using the WinNonlin® software with noncompartment model analysis.
Results: The mean ± SD of maximum plasma concentration (C_{max}), the area under the plasma-concentration time curve from 0 to 48 h (AUC_{0-48}) and the area under the plasma-concentration time curve from 0 to infinity ($AUC_{0-\infty}$) of Quantia 200® v.s. Seroquel® were 886.60 ± 356.50 v.s. 811.34 ± 323.37 ng/ml; 3,754.41 ± 1,453.00 v.s. 3,420.00 ± 1,229.6 ng.h/ml and 4,015.35 ± 1,528.25 v.s. 3,769.45 ± 1,296.69 ng.h/ml, respectively. Time to reach C_{max} (T_{max}) of Quantia 200® and Seroquel® were 1.08 ± 0.778 and 1.10 ± 0.79 h., respectively and were not significantly different. The 90% confidence interval of the ratios of the logarithmically transformed of C_{max}, AUC_{0-48} and $AUC_{0-\infty}$ were 98.21-124.37%, 94.43-117.03% and 94.77-116.61%, respectively, which were within the acceptable range of 80-125%. Power of the test for C_{max}, AUC_{0-48} and $AUC_{0-\infty}$ were 92.1%, 96.9% and 97.4%, respectively.
Conclusion: Quantia 200®, used in this study, was bioequivalent to Seroquel® in terms of both the rate and extent of absorption.
Keywords : Bioequivalence, pharmacokinetics, quetiapine, Quantia 200®, Seroquel®

P19 Bioequivalence Study of Gabapentin 300 mg Capsule in Thai Healthy Volunteers

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Abstract

The objective of this study is to compare the bioavailability of new generic product of gabapentin with the innovator's product. The study was performed in 20 Thai male healthy volunteers who received a single oral dose of 300 mg gabapentin. Double blind randomized two way crossover design was used with one week washout period between treatments. After drug administration, serial blood sample was collected over a period of 32 hours. Plasma gabapentin was determined by automated High Performance Liquid Chromatography (HPLC) with fluorescence detection after deproteinized with acetonitrile and following derivatization with o-phthaldehyde (OPA) reagent containing 2-mercaptoethanol. The difference of pharmacokinetic parameters, Cmax, $AUC_{(0-t)}$ and $AUC_{(0-inf)}$ were analyzed by Two Way Analysis of Variance (ANOVA) and 90% confidence interval. The maximum concentration (C_{max}, μg/ml) of gabapentin was 3.26±0.60 (range 1.58-4.07) and 3.00±0.71 (range 1.42-3.95) μg/ml for generic and innovator's product, respectively. The time to peak plasma gabapentin concentration (T_{max}, hr) of generic and innovator's product was 3.08±0.59 (2-4) and 3.33±0.63 (2-5), respectively. The area under the plasma concentration-time curve of generic and innovator's product were 30.06±4.94 vs 27.63±6.45 μg.hr/ml for $AUC_{(0-t)}$ and 30.76±4.88 vs 28.27±6.63 μg.hr/ml for $AUC_{(0-inf)}$, respectively. 90% CI of C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-inf)}$ of generic compared to innovator's product were 96.64-124.94%, 97.60-124.43% and 97.90-124.00%, respectively. They were within the acceptance range of 80-125 %, thus we concluded that gabapentin from two formulations are bioequivalent.

Keywords: Bioequivalence, pharmacokinetics, gabapentin

P20 Inhibition of Neutrophil Functional Responsiveness and T-Lymphocyte Proliferation by (-) Panduratin-A

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Abstract

Introduction: (-) Panduratin A, a cyclohexenyl chalcone derivative isolated from the chloroform extract of the red rhizome variety of *Boesenbergia pandurata* (Robx) Schltr, showed significant topical anti-inflammatory activity in the TPA-induced ear edema in rats.
Objective: We aimed to investigate the effects of (-) Panduratin-A on human neutrophil responsiveness and T-lymphocyte proliferation to elucidate underlying cellular mechanisms of its powerful anti-inflammatory activity.
Materials and methods: Human neutrophil responsiveness was determined by measuring fMLP-induced chemotaxis, superoxide anion generation (SAG), and release of MPO and elastase. Apoptosis was assessed morphologically and flow-cytometrically. Neutrophil viability was assessed by trypan blue exclusion and XTT cytotoxicity assays. T lymphocyte proliferation was quantified by [^{3}H] thymidine incorporation.
Results: Our results showed that treatment of neutrophils with (-) Panduratin-A concentration-dependently inhibited fMLP-induced chemotaxis, SAG, and MPO and elastase release, although Panduratin-A did not affect neutrophil viability or apoptosis. Panduratin-A was also inhibited T lymphocyte proliferation as quantified by [^{3}H] thymidine incorporation.
These findings suggest that inhibition of neutrophil functional responsiveness and T-lymphocyte proliferation may be attributed, in part, to the powerful anti-inflammatory properties of (-) Panduratin-A .

Keywords: Boesenbergia pandurata; *(-)panduratin A* ; Neutrophil functional responsiveness; T-lymphocyte proliferation

P21 Neuroprotective Effects of Curcumin on Hydrogen peroxide-induced Neurotoxicity in SH-SY5Y Human Neuroblastoma Cells

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Abstract

Curcumin, the major yellow pigment in turmeric (*Curcuma longa*), is a naturally-occurring anti-oxidant with numerous pharmacological activities such as anti-inflammatory, anti-carcinogenic and anti-bacterial effects. Oxidative stress plays an important role in the pathological processes of a variety of neurodegenerative diseases. The objective of the present study was to study the protective effects of curcumin on the survival of SH-SY5Y human neuroblastoma cells (SH-SY5Y cells) in the presence of hydrogen peroxide (H_2O_2). SH-SY5Y cells treated with H_2O_2 exhibited a decrease in survival. However, pretreatment of cells with curcumin attenuated the neuronal death induced by H_2O_2. The data suggest that curcumin might be a potential therapeutic agent for treating or preventing neurodegenerative diseases implicated with oxidative stress.

Keywords: curcumin, antioxidant, SH-SY5Y human neuroblastoma cells

Introduction

Curcumin, the yellow pigment from the rhizoma of *C.longa*, is a widely studied phytochemical which has a variety of biological activities: anti-inflammatory and anti-oxidative [1]. Oxidative stress play a key role in aging, it is not surprising that diet supplementation with antioxidants may increase life span. Recent data suggest that curcumin and other antioxidant products from the dried rhizome of *C.longa* may be useful for the prevention and / or treatment of some age-related degenerative processes. SH-SY5Y cells were exposed to oxidative stress induced by H_2O_2, which has been extensively used to induce reactive oxygen species (ROS) productions. In this study the protective effect of curcumin on hydrogen peroxide (H_2O_2)-induced neuronal cell damage was evaluated.

Materials and Methods

1. Materials

Human SH-SY5Y cell was purchased from American Type Culture Collection, UK. Curcumin and common laboratory chemicals were obtained from Sigma, St. Louis, MO.

2. Culture of SH-SY5Y cells

SH-SY5Y cells were grown in equal parts of Minimum Essential Medium (MEM) with Earle's Salts and nutrient mixture Ham's F-12 supplemented with nonessential amino acid, pyruvate and 10 % heat-inactivated fetal bovine serum. At confluence, the cells were harvested and seeded onto culture plates for further experiments [2].

3. Cell viability assay

SH-SY5Y cells were cultured onto 96-well plate at a density of $2x10^4$ cells/well overnight. The cells were then treated with various chemicals according to the experimental design. After 24 h the medium was removed and 10 µl of 0.25 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and further incubated

for 4 h in a humidified atmosphere at 37°C, 5% CO_2. MTT was removed and cells were lysed with 100 µl DMSO, then the absorbance was measured at 570 nm and at a reference wavelength of 665 nm on a microplate reader [3].

4. Detection of ROS by using 2□,7□-dichlorofluorescein (DCHF-DA) and dihydrorhodamine 123 (DHR)

SH-SY5Y cells were plated at a density of $2x10^4$ viable cells per well in 96-wells fluorescent plate. Cells were grown in culture medium and incubated at 37°C. Following 48 h incubation, cells were washed once with PBS pH 7.4 before loading with freshly prepared of 10 µM DCFH-DA or DHR in HBSS for 1 h at 37°C. After the loading period, cells were washed with HBSS and treated with HBSS containing H_2O_2 in the presence or absence of curcumin. Increases in fluorescence were measured immediately at 37°C for 15 min interval up to 6 h. Measurement of developing fluorescent intensity is performed every 15 min for 2 h at 37□C by using automated microplate reader with filters 485/510 nm for DHR and 485/535 nm for DCFH-DA [4].

5. Statistics

All data were indicated as the mean ± SEM of *n* experiments. Statistical analysis was performed by a one-way analysis of variance (ANOVA). Values of p_0.05 were considered statistically significant.

Results

The SH-SY5Y cell was used as a neuronotypic model of H_2O_2-induced cell death. H_2O_2 causes a dose-dependent decrease in cell viability in SH-SY5Y cells, as measured by the reduction of MTT. Figure 1 showed the effects of H_2O_2 on cell viability. At 6 h of 600 µM H_2O_2 incubation markedly reduced the cell survival 40%. The curcumin at concentration 100 µM did not affect cell survival up to 6 h. Pretreatment of cells with 100 µM curcumin attenuated the neuronal death induced by H_2O_2.

In order to determine the mechanism of the neuroprotective effect of curcumin on oxidative stress induced toxicity; the level of free radical production was detected. The level of intracellular ROS using 5, 6-carboxy-2',7'-dihydrodichloro-fluorescein diacetate (DCFH-DA) fluorescent dye, and mitochondrial ROS using tetramethyl-rhodamine methyl ester (DHR) fluorescent dye were assessed in SH-SY5Y cells. Figure 2 showed the time course of changes in cellular fluorescence in response to 600 µM H_2O_2 in SH-SY5Y cells. H_2O_2 caused rapidly increased the intracellular ROS production during 6 h. The mitochondrial ROS formations were showed in Figure 3. Curcumin significantly inhibited intracellular and mitochondrial ROS production in the presence and absence of H_2O_2.

Discussion

The neuroprotective effect of curcumin was investigated in this study by using an *in vitro* model of the SH-SY5Y cells. The present study revealed that curcumin could inhibit the formation of intracellular and mitochondrial ROS in the control condition and under oxidative stress induced by H_2O_2. H_2O_2 is a water soluble hydroperoxide that diffuses easily across cellular compartments. The toxic effect of H_2O_2 involved its ready conversion to reactive hydroxyl radical. Normally, the ROS is produced in several physiological processes, which further interact with other molecules to accelerate the ageing phenomena due to the damaging consequences of free radical action. The 100 µM curcumin had no cytotoxic effect

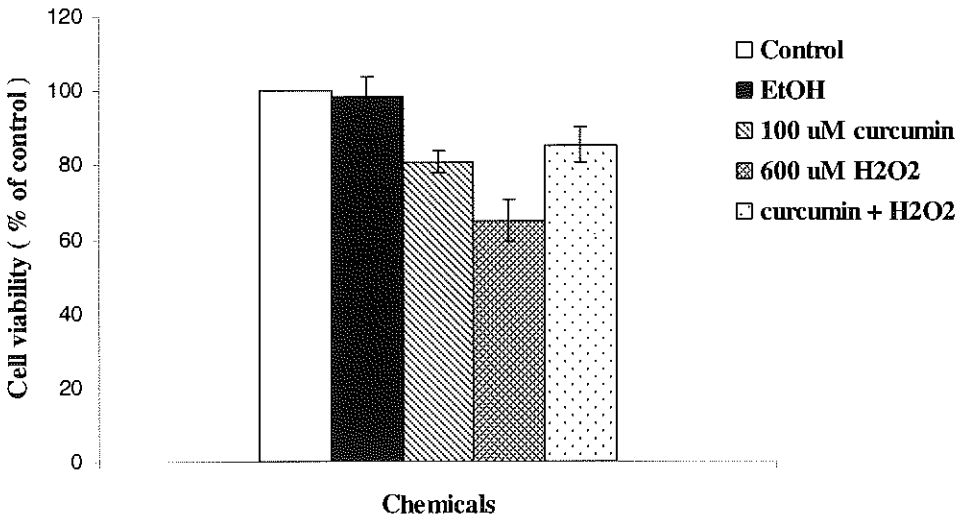

Figure 1 The Effect of curcumin on H_2O_2-induced toxicity. SH-SY5Y cells were exposed to 600 μM H_2O_2 for 6 hours in the absence or presence of 100 μM curcumin. Cell viability was determined by MTT assay. Data are presented as the percent of the untreated cell. Values represent mean ± SEM of three experiments, each performed in quadruplicate. * $P < 0.05$ compared with the cells treated only with H_2O_2.

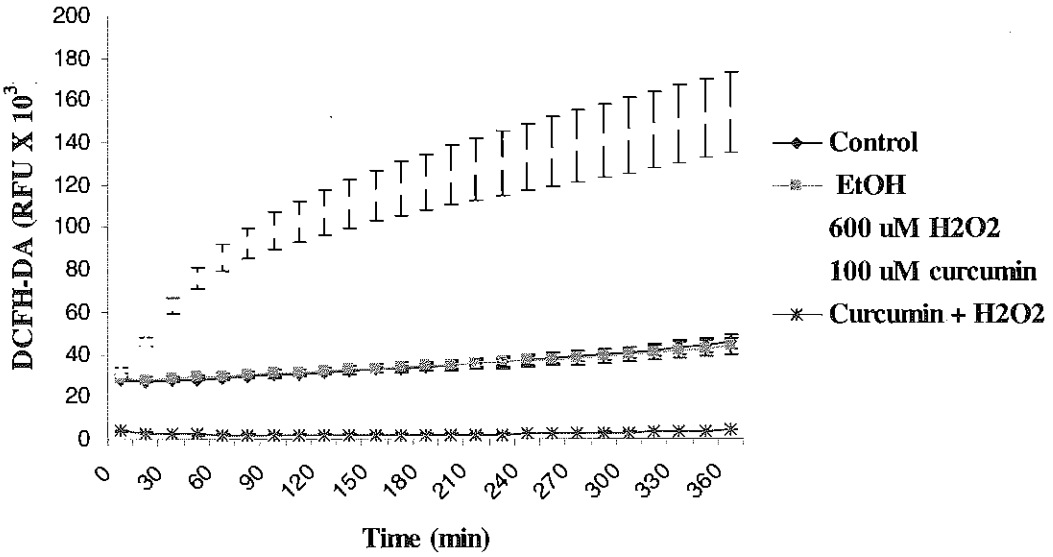

Figure 2 Time course of H_2O_2 induced free radical formation. SH-SY5Y cells were treated with 600 μM H_2O_2. Intracellular free radical production was monitored by measurement of DCF fluorescence. Data are presented as the percent of the untreated cell. Values represent a mean ± SEM of three experiments, each performed in quadruplicate.

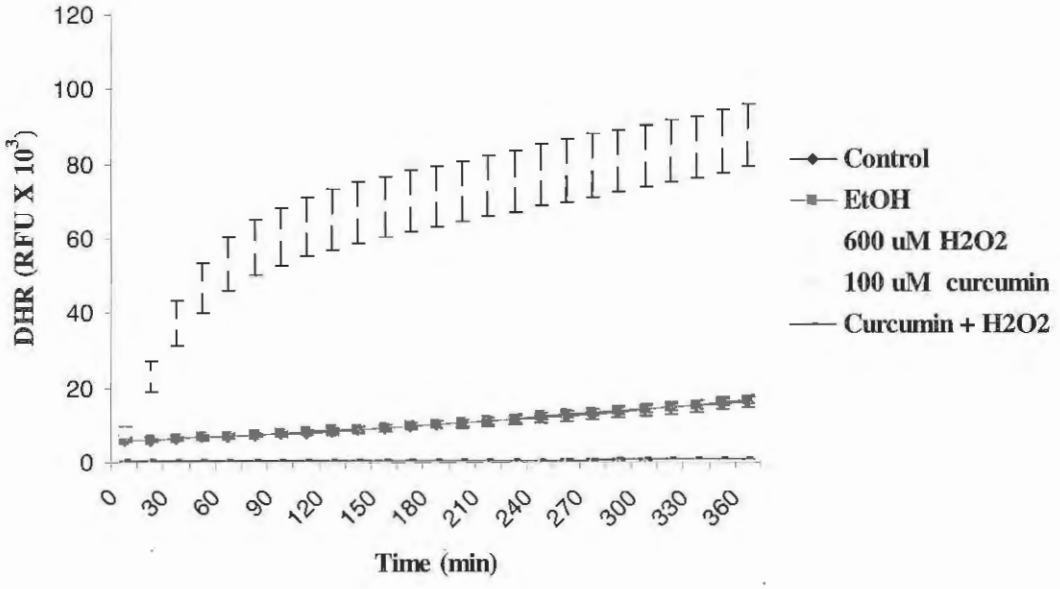

Figure 3 Time course of H_2O_2 induced free radical formation. SH-SY5Y cells were treated with 600 μM H_2O_2. Mitochondrial free radical production was monitored by measurement of DHR fluorescence. Data are presented as the percent of the untreated cell. Values represent a mean ± SEM of three experiments, each performed in quadruplicate.

and possessed neuroprotection from H_2O_2-induced toxicity by the reduction of free radical accumulation in the cytoplasm and mitochondria. Therefore, curcumin may be benefit on the retardation of the ageing process as claimed in the benefits of curcumin in the traditional medicine.

Conclusion

Curcumin could protect SH-SY5Y cells against H2O2–induced oxidative stress in both cytoplasm and mitochondria. The current study revealed that curcumin could exhibit neuroprotection in SH-SY5Y neuroblastoma cells by theirs antioxidant effect.

Acknowledgement

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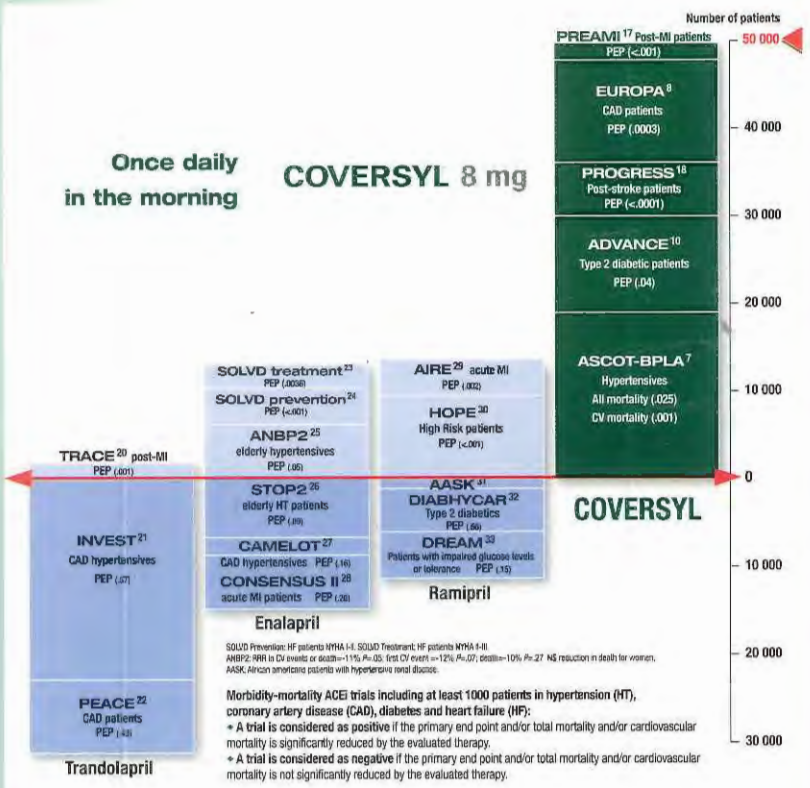

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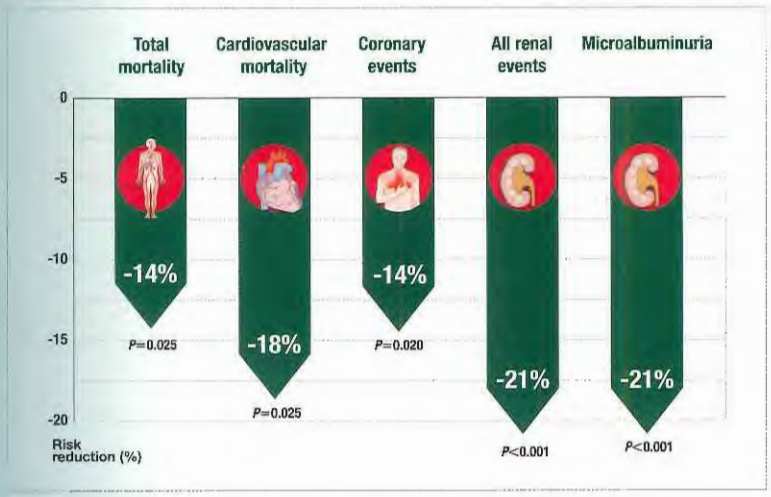
ADVANCE

ACTION IN DIABETES AND VASCULAR DISEASE: PRETERAX AND DIAMICRON MR CONTROLLED EVALUATION

"The results of ADVANCE should have MAJOR IMPLICATIONS for guidelines, clinical care, and public policies"

Prof MacMahon

Primary end point: macrovascular + microvascular outcomes *P*=0.041



"The fixed dose combination regimen used in ADVANCE was well tolerated." During the prerandomization period, only 3.6% of patients were withdrawn because of side effects. "Adherence was comparable to that seen with placebo during the 4.3 years of the trial." "This result has important practical implications for health services delivery, since only one follow-up visit is needed to established a patient's suitability for long-term treatment with this regimen."

ADVANCE Collaborative Group. *Lancet.*

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