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ระหว่างวันที่ 21-23 มีนาคม 2550
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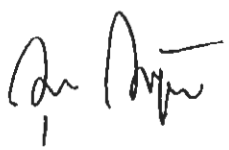
สารจากนายกสมาคมเภสัชวิทยาแห่งประเทศไทย

เรียน ท่านสมาชิกสมาคมเภสัชวิทยาและผู้เข้าร่วมประชุมทุกท่าน

ขอต้อนรับทุกท่านเข้าร่วมประชุมวิชาการประจำปีครั้งที่ 29 ของสมาคมเภสัชวิทยาแห่งประเทศไทย การประชุมครั้งนี้สมาคมฯ ได้รับความร่วมมือจากภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล ในการเป็นเจ้าภาพร่วมและได้พิจารณาจัดการประชุมในหัวข้อเป็นที่น่าสนใจ มีสาระที่เหมาะสมสอดคล้องกับการเปลี่ยนแปลงอย่างมากทางวิทยาศาสตร์และการพัฒนาความรู้ทางเภสัชวิทยาอย่างรวดเร็วตลอดทศวรรษที่ผ่านมา เช่น การจัดให้มีการเสวนาด้านการเรียนการสอนเภสัชวิทยา ตลอดถึงแนวทางการประเมินความรู้ของนักศึกษาแพทย์ในแนวทางของหลักสูตรเชิงบูรณาการ โดยผู้เชี่ยวชาญทางแพทยศาสตร์ศึกษา และเกณฑ์ประเมินความรู้นักศึกษาแพทย์ตามเกณฑ์ของทางแพทยสภา มีการทบทวนความเป็นปัจจุบันของกลุ่มยารักษาโรคซึมเศร้า กลุ่มยารักษาโรคหัวใจและหลอดเลือด จากความเข้าใจขณะนี้เกี่ยวกับกลไกทางพยาธิสภาพของโรค การเสนองานวิจัยด้านสมุนไพร เพื่อวางแนวทางการวิจัยร่วมเชิงบูรณาการร่วมกัน มุ่งสู่แนวทางที่เหมาะสมในการพัฒนายาใหม่จากสมุนไพร

สำหรับปาฐกถาพิเศษ รศ. จิรวัฒก์ สดาวงศ์วิวัฒน์ "ครั้งที่ 14" สมาคมฯ ได้รับเกียรติจาก Professor Graeme Henderson นายกสมาคมเภสัชวิทยาแห่งประเทศสหราชอาณาจักรมาเป็นองค์ปาฐกในครั้งนี้ ในหัวข้อ เรื่อง กลไกการดื้อยาของกลุ่มยาแก้ปวดชนิดเสพติด (opioids) ผ่าน μ-receptor ทำให้การประชุมวิชาการประจำปีครั้งนี้มีความสำคัญยิ่งครั้งหนึ่ง

ในนามของสมาคมฯ กระผมใคร่ขอขอบคุณวิทยากร นักศึกษา คณะกรรมการจัดการประชุมฯ หน่วยงานภาคเอกชน ที่ร่วมให้การสนับสนุน และผู้เข้าร่วมประชุมทุกท่านที่ได้มีส่วนทำให้การประชุมครั้งนี้ สำเร็จได้ผลเป็นที่พอใจทุกประการ



ดร. อุดม จันทรารักษ์ศรี

นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

สารจากประธานจัดงานประชุมวิชาการประจำปีครั้งที่ 29

เรียน ท่านสมาชิกชาวเภสัชวิทยาและผู้เข้าร่วมประชุมทุกท่าน

การประชุมวิชาการประจำปีของสมาคมเภสัชวิทยาแห่งประเทศไทย เป็นกิจกรรมหลักของสมาคมฯ เพื่อเป็นเวทีสำหรับติดตามความก้าวหน้าของวิชาการสาขาเภสัชวิทยา แลกเปลี่ยนความรู้ความคิดเห็นและประสบการณ์ระหว่างนักเภสัชวิทยาและนักวิชาการสาขาอื่นที่เกี่ยวข้อง นอกจากนี้ยังเป็นโอกาสอันดีที่สมาชิกและผู้สนใจจะได้พบปะสังสรรค์ เชื่อมความสามัคคี อันจะนำไปสู่ความร่วมมือด้านการเรียนการสอนและการวิจัยในอนาคต เพื่อให้สถาบันที่จัดการเรียนการสอนและการวิจัยทางเภสัชวิทยามีความเข้มแข็งและก้าวไปด้วยกันฉันท์เพื่อน-พี่-น้อง และทำให้วิชาการสาขาเภสัชวิทยาในประเทศไทยมีความก้าวหน้าและมีความทันสมัยเป็นที่ยอมรับในระดับสากล

ดิฉันใคร่ขอแสดงความขอบคุณเท่านผู้เข้าร่วมประชุมทุกท่าน วิทยากร และผู้ให้การสนับสนุนทุกฝ่าย หากมีข้อผิดพลาดและข้อบกพร่องประการใด ที่อาจเกิดขึ้น ดิฉันต้องขออภัยไว้ล่วงหน้า และขอรับคำแนะนำด้วยความยินดี

รศ. ดร. ยุพิน สังวรินทะ
ประธานกรรมการจัดประชุมฯ

คำปราศรัยของ ศ. ดร. อมเรศ ภูมิรัตน
คณบดีคณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
เนื่องในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 29
สมาคมเภสัชวิทยาแห่งประเทศไทย
ในวันพฤหัสบดีที่ 22 มีนาคม 2550

ท่านประธานคณะกรรมการจัดประชุมฯ ท่านนายกสมาคมเภสัชวิทยาแห่งประเทศไทย และท่านผู้มีเกียรติทุกท่าน

กระผมรู้สึกเป็นเกียรติและยินดีอย่างยิ่ง ที่ได้มีโอกาสมาเป็นประธานในพิธีเปิดการประชุมวิชาการประจำปี ครั้งที่ 29 ของสมาคมเภสัชวิทยาแห่งประเทศไทยในวันนี้ ขอแสดงความยินดีที่สมาคมเภสัชวิทยาฯ ได้ก่อตัวและประสบความสำเร็จในการดำเนินงานมาโดยตลอด

ปัจจุบัน วิชาการสาขาต่างๆ และเทคโนโลยีได้ก้าวหน้าไปอย่างรวดเร็ว โดยเฉพาะอย่างยิ่งการวิจัยและพัฒนายาใหม่ๆ ออกสู่ท้องตลาดเป็นจำนวนมาก และโดยที่ยุคนี้จัดว่าเป็นยุคโลกไร้พรมแดน การประชุมวิชาการเป็นวิธีการหนึ่งที่ทำให้นักวิชาการสามารถติดตามรับทราบข้อมูลวิชาการและเทคโนโลยีที่ก้าวรุดหน้าได้รวดเร็วขึ้น ซึ่งจะทำให้นักเภสัชวิทยาและบุคลากรด้านการแพทย์ได้พัฒนาตนเอง เป็นประโยชน์ต่อทั้งหน่วยงานและประเทศชาติ

บัดนี้ได้เวลาอันสมควรแล้ว กระผมขอเปิดการประชุมวิชาการครั้งที่ 29 ของสมาคมเภสัชวิทยาแห่งประเทศไทย และขออวยพรให้การประชุมครั้งนี้บรรลุตามวัตถุประสงค์ และสำเร็จสมดังเจตนารมณ์ที่ตั้งไว้ทุกประการ

ศ. ดร. อมเรศ ภูมิรัตน
คณบดีคณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

บรรณาธิการแถลง

เรียนท่านผู้เข้าร่วมประชุมและสมาชิกสมาคมเภสัชวิทยาแห่งประเทศไทย

วารสารฉบับนี้จัดทำขึ้นเพื่อเป็นเอกสารประกอบการประชุมวิชาการประจำปี 2550 ของสมาคมเภสัชวิทยาแห่งประเทศไทย ในปีนี้จะเป็นครั้งแรกที่เนื้อหาของผลงานที่นำเสนอจะจัดทำเป็นรูปแบบที่ให้รายละเอียดมากขึ้นนอกเหนือจากการใส่แค่เพียงบทคัดย่อดังที่ผ่านมา

การประชุมในปีนี้ก็คงเช่นเดียวกับทุกๆปีที่ผ่านมาที่เนื้อหายังคงอยู่ในประเด็นที่กำลังเป็นที่น่าสนใจ ไม่ว่าจะเป็นรูปแบบการรักษาโรคที่พัฒนาไปตามกระแสโลกในยุคโลกาภิวัฒน์ ซึ่งแน่นอนว่าโรคในระบบประสาทที่ค่อนข้างซับซ้อนตามโครงสร้างทางสังคมที่ซับซ้อนกว่าในอดีต และโรคที่สะท้อนถึงภาวะโภชนาการเช่นโรคที่เกี่ยวข้องในระบบหัวใจและหลอดเลือด การเสาะแสวงหาแนวทางพัฒนายาใหม่ก็สอดคล้องกับความต้องการและปัญหาของสังคมในแต่ละยุคสมัย ราวกับเป็นแฟชั่นของการรักษาและการพัฒนายา

ตามหัวข้อของการจัดงานน่าจะมีส่วนเสริมความรู้ที่ทันยุคทันเหตุการณ์ และยังได้แลกเปลี่ยนความคิดเห็นในงานวิจัยแสวงหายาจากทรัพยากรธรรมชาติของประเทศ สำหรับผู้ที่พลาดจากการประชุมวิชาการในครั้งนี้ยังสามารถติดตามได้จากวารสารฉบับพิเศษฉบับนี้ได้ตามปกติดังที่ผ่านมาในทุกปี

ขอขอบพระคุณภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดลที่รับเป็นเจ้าภาพในการจัดงานและให้ความร่วมมืออย่างแข็งขันในการจัดทำวารสารที่เป็นเอกสารประกอบการประชุมวิชาการฉบับนี้

รศ. ดร. สุพัตรา ศรีไชยรัตน์
บรรณาธิการ

สรุปผลงานคณะกรรมการบริหารสมาคมเภสัชวิทยาแห่งประเทศไทย

วาระ 2549-2551

มีนาคม 2549-มีนาคม 2550

1. จัดประชุมวิชาการ เรื่อง "ผลกระทบต่อสุขภาพของสารต้านอนุมูลอิสระและแคฟเฟอีนในกาแฟ" โดย รศ.ดร.ชัยชาญ แสงดี ในวันจันทร์ที่ 11 กันยายน 2549 ณ โรงแรมปทุมวันพริ้นเซส กรุงเทพฯ
2. จัดงานแสดงมุทิตาจิตแด่อาจารย์เกษียณอายุราชการ ในวันจันทร์ที่ 18 กันยายน 2549 เวลา 11.00 – 13.00 น. ณ ห้อง ทับทิมสยาม สโมสรกองทัพบก
3. จัดประชุมวิชาการร่วมกับสถาบันการศึกษาต่อเนื่องทางเภสัชศาสตร์ สำนักงานคณะกรรมการอาหารและยา เพื่อการบริการวิชาการแด่บุคลากรทางด้านสาธารณสุข ณ สำนักงานคณะกรรมการอาหารและยาในเรื่อง

3.1 "การใช้ยาเพื่อบำบัดผู้ติดยาเสพติด" โดย พ.อ.รศ.ดร.บพิตร กลางกัลยา ในวันศุกร์ที่ 22 กันยายน 2549 เวลา 10.00 – 12.00 น.

3.2 "ความรู้เกี่ยวกับภูมิคุ้มกันร่างกายในยุคดิจิตัล : Therapeutic Antibodies" โดย ผศ.ดร.วัชรี ลิมปนสิทธิกุล ในวันพุธที่ 20 ธันวาคม 2549 เวลา 09.00 – 12.00 น.

3.3 "การสืบค้นข้อมูลด้านยาและการนำไปประยุกต์ : Evidence search in drug information and its application" โดย ผศ.นพ.พิสนธิ์ จงตระกูล ในวันพฤหัสบดีที่ 22 กุมภาพันธ์ 2550 เวลา 9.30 –12.00 น.

4. จัดประชุมวิชาการเรื่อง "Pharmacology in Post-genomic Era" โดย ศ.นพ.บุญส่ง องค์พิพัฒน์กุล ศ.นพ.สุทัศน์ ฟู่เจริญ ศ.นพ.ประสิทธิ์ ผลิตผลการพิมพ์ และ ดร.อุดม จันทรารักษ์ศรี ผู้ดำเนินการอภิปราย ในวันพฤหัสบดีที่ 19 ตุลาคม 2549 เวลา 09.00 – 16.00 น. ณ ห้อง K102 ตึกเฉลิมพระเกียรติ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
5. สืบเนื่องจากการประชุม "Pharmacology in Post-genomic Era" จึงได้มีการจัดสัมมนา Series of Pharmacology in Post-genomic Era อย่างต่อเนื่องทุกวันศุกร์ที่สองของเดือน ณ ห้องอัลเบิร์ต คุปเปอร์แมน ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล โดยเน้นการสัมมนาแก่นิสิตบัณฑิตศึกษา และคณาจารย์ หัวข้อเรื่องของการสัมมนาที่ได้ดำเนินการแล้ว มีดังนี้

5.1 Research questions in pharmacogenomics โดย ดร.อุดม จันทรารักษ์ศรี ในวันศุกร์ที่ 10 พฤศจิกายน 2549 เวลา 14.00-16.00 น.

5.2 Genomic research โดย ศ.นพ.สุทัศน์ ฟู่เจริญ ในวันศุกร์ที่ 8 ธันวาคม 2549 เวลา 14.00-16.00 น.

5.3 Strategic gene search โดย วัฒนันท์ มกรสาร ในวันศุกร์ที่ 9 มกราคม 2550 เวลา 14.00-16.00 น.

5.4 Pharmacogenomics in the SNP Era โดย รศ. ดร.วสันต์ จันทราทิตย์ ในวันศุกร์ที่ 9 กุมภาพันธ์ 2550 เวลา 14.00-16.00 น.

6. จัดประชุมปรึกษาหารือเกี่ยวกับ"แนวทางการจัดการเรียนการสอนให้สอดคล้องกับความต้องการของแพทยสภาและแนวทางการจัดทำข้อสอบศรว."ในวันจันทร์ที่ 18 ธันวาคม 2549 เวลา 13.00 – 16.00 น. ณ ห้องK101 ตึกเฉลิมพระเกียรติ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

7. จัดทำโครงการเผยแพร่ข้อมูลผ่านทางเว็บไซต์สมาคมเภสัชวิทยาแห่งประเทศไทย www.phartherst.org

8. จัดทำจุลสารสมาคมเภสัชวิทยาแห่งประเทศไทยผ่านเว็บไซต์สมาคมฯ www.phartherst.org

- ฉบับที่ 1/2549 ประจำเดือน ตุลาคม 2549
- ฉบับที่ 2/2549 ประจำเดือน พฤศจิกายน 2549
- ฉบับที่ 3/2549 ประจำเดือน ธันวาคม 2549
- ฉบับที่ 1/2550 ประจำเดือน มกราคม 2550

9. จัดทำวารสารสมาคมเภสัชวิทยาแห่งประเทศไทย เล่มที่ 28 วันที่ 20 พฤษภาคม 2549 หัวข้อ "Clinical pharmacology for hospital pharmacists "

10. สร้างกลุ่มเครือข่ายวิชาการสมาคมเภสัชวิทยาแห่งประเทศไทย เพื่อการพัฒนางานด้านวิชาการและเผยแพร่ข้อมูลข่าวสารด้านเภสัชวิทยา แก่คณาจารย์ นิสิตบัณฑิตศึกษา สมาชิก และผู้สนใจ

11. จัดทำคลังข้อมูล e-mail address ของสมาชิก นิสิตบัณฑิตศึกษา และกลุ่มเครือข่ายวิชาการสมาคมฯ

รายนามวิทยากร

Professor Graeme Henderson President of the British Pharmacological Society	Department of Pharmacology, University of Bristol, Bristol, UK
รศ.นพ. รณชัย คงสกนธ์	ภาควิชาจิตเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี
ศ.ดร. ปิยะรัตน์ โกวิทตรพงศ์	หน่วยวิจัยประสาทวิทยาศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
รศ.ดร. จุฑามณี สุทธิสีสังข์	ภาควิชาเภสัชวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
รศ.ดร. พรทิพย์ ศุภวิไล	ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
รศ.นพ. ปิยะมิตร ศรีธรา	ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี
รศ.นพ. นิพนธ์ ฉัตรทิพากร	ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
ผศ.ดร. พยงค์ วณิเกียรติ	ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
ศ. นันทวัน บุณยะประภัศร	ภาควิชาเภสัชวินิจฉัย คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
ศ.ดร. ภาวิณี ปิยะจตุรวัฒน์	ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
รศ.ดร. สุภา หารหนองบัว	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์
ดร. ฉัตรชัย เหมือนประสาท	ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

กำหนดการประชุมวิชาการประจำปี ครั้งที่ 29
สมาคมเภสัชวิทยาแห่งประเทศไทย
ร่วมกับ ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

วันที่ 21-23 มีนาคม 2550

ณ ห้อง K 102 อาคารเฉลิมพระเกียรติ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล และ ห้องสดศรี วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

Pre-meeting: Pharmacology Teaching

วันพุธที่ 21 มีนาคม 2550 ณ ห้อง K 102 อาคารเฉลิมพระเกียรติ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

9.00 - 10.15 น.	การเรียนการสอนเภสัชวิทยาเชิงบูรณาการ โดย อ.นพ.คร. ดนัย วังสตุรค
10.15 - 10.30 น.	พัก - น้ำชา กาแฟ
10.30 - 12.00 น.	การสอนเภสัชวิทยาในมุมมองของแพทยสภา โดย รศ.นพ.อานุภาพ เลขะกุล
12.00 - 13.00 น.	รับประทานอาหารกลางวัน
13.00 - 14.30 น.	แนวคิดการออกข้อสอบเภสัชวิทยาแบบบูรณาการของ ศ.ร.ว. โดย รศ.นพ.อานุภาพ เลขะกุล
14.30 - 14.45 น.	พัก – น้ำชา กาแฟ
14.45 - 16.00 น.	อภิปรายรวม เรื่อง ทิศทางการสอนและการออกข้อสอบวิชาเภสัชวิทยา โดย ผู้แทนจากภาควิชาเภสัชวิทยาของแต่ละสถาบัน ผศ.นพ.คร.วิทยา ตันสุวรรณนนท์ ผู้ดำเนินการอภิปราย

Frontiers in Pharmacology: Challenging for better therapeutic approach

วันพฤหัสบดีที่ 22 มีนาคม 2550 ณ ห้องสดศรี วิทยาลัยแพทยศาสตร์พระมุงกุฎเกล้า

08.00-08.30	ลงทะเบียน
08.45-09.15	ประธาน กล่าวรายงาน พิธีเปิดการประชุม โดย คณบดีคณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล นายกสมาคมฯ กล่าวต้อนรับผู้เข้าร่วมประชุม
09.15-10.45	The 14th Dr. Chiravat Sadavongvivad Memorial Lecture: Agonist-Selective Mechanisms of mu-Opioid Receptor Desensitization: Roles for PKC and GRK By Professor Graeme Henderson Department of Pharmacology, University of Bristol, Bristol UK President of the British Pharmacological Society ผู้ดำเนินรายการ : ผศ. คร. พยงค์ วณิเกียรติ

10.45-11.15	พัก-อาหารว่างและเครื่องดื่ม
11.15-12.00	ประชุมธุรการสมาคมฯ
12.00-13.00	Luncheon Seminar: Neuroplasticity with Antidepressant วิทยากร : รศ.นพ. รณชัย คงสกนธ์ ประธาน : รศ. ดร. ยุพิน สังวรินทะ
13.15-14.45	Symposium I: Drugs in Neurodegenerative Diseases วิทยากร : รศ.ดร. จุฑามณี สุทธิสีสังข์ ศ.ดร. ปิยะรัตน์ โกวิทตรพงศ์ รศ.ดร. พรทิพย์ ศุภวิไล
14.45-15.45	Oral and Poster Presentation ผู้ดำเนินรายการ : รศ.ดร. สมใจ นครชัย รศ.ดร. กรองทอง ยุวถาวร
15.45-16.00	พัก-อาหารว่างและเครื่องดื่ม
17.30-19.30	Welcome Dinner

วันศุกร์ที่ 23 มีนาคม 2550 ห้องสดศรี วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

9.00-9.45	Plenary Lecture I: Cardiovascular Diseases: Nature Burden in Thai Population วิทยากร : รศ.นพ. ปิยะมิตร ศรีธรา ประธาน : รศ. ดร. ศรีจันทร์ พรจิราศิลป์
09.45-10.00	พัก-อาหารว่างและเครื่องดื่ม
10.00-12.00	Symposium II: Advances in Arrhythmic Death and Intervention วิทยากร : รศ.นพ. นิพนธ์ ฉัตรทิพากร ผศ.ดร. พยงค์ วณิเกียรติ
12.00-13.00	อาหารกลางวัน
13.00-13.45	Plenary Lecture II: สกว. กับงานวิจัยสมุนไพร วิทยากร : ศ. ดร. นันทวัน บุณยะประภัศร ประธาน : ดร. อุดม จันทรารักษ์ศรี
13.45-15.15	Symposium III: Herbal Based Drug Discovery วิทยากร : ศ.ดร. ภาวิณี ปิยะจตุรวัฒน์ รศ.ดร. สุภา หารหนองบัว ดร. ฉัตรชัย เหมือนประสาท
15.15-15.30	พัก-อาหารว่างและเครื่องดื่ม
15.30-16.00	ประกาศ ผลการประกวดผลงานวิจัย พิธีปิดการประชุมโดยนายกสมาคมฯ

Chiravat Memorial Lecture

Agonist-Selective Mechanisms of μ-Opioid Receptor Desensitisation: Roles for PKC and GRK

Prof. Graeme Henderson

Department of Pharmacology, University of Bristol, Bristol UK.

The analgesic and euphoric effects of morphine occur through activation of μ-opioid receptors (MORs). In the intact animal tolerance to morphine, defined as the process whereby continued use of morphine results in the requirement of an increased dose to produce equivalent effect, can occur rapidly. The mechanisms underlying morphine tolerance are not well understood, but it is thought to involve changes at the level of the MORs. MORs, like other G-protein-coupled receptors, rapidly desensitise in response to various opioid agonists. However, there is considerable controversy over whether the prototypic MOR ligand, morphine, induces significant MOR desensitisation and this has called into question the role of MOR desensitisation in morphine tolerance. In rat brainstem neurons morphine produces significantly less receptor desensitisation than other MOR agonists whereas in certain recombinant systems morphine can produce profound MOR desensitisation (for review see Bailey et al 2006).

Recently we have suggested that the level of PKC activity within a cell is critical for observing morphine-induced MOR desensitisation in both mature neurons and HEK-293 cells. We have demonstrated that different opioid agonists can induce MOR desensitisation by different intracellular mechanisms. Thus, the high efficacy peptide agonist DAMGO induced rapid desensitisation through a G-protein coupled receptor kinase (GRK) dependent mechanism whereas morphine induced desensitisation is largely mediated by PKC. Using both novel, specific PKC isoform inhibitors and PKC knockout mice we have identified the PKC isoform responsible for morphine-induced MOR desensitisation in mature brainstem locus coeruleus (LC) neurons as PKCα. We have also used an adenoviral vector to over-express a GRK dominant negative mutant (DNM) in mature LC neurons and have found that DAMGO-induced, but not morphine-induced desensitisation, is attenuated.

These results demonstrate that the mechanisms underlying MOR desensitisation in mature neurons are determined by the agonist activating the receptor.

Bailey et al., (2006) Trends in Pharmacological Sciences 27, 558-565

S1-1 Melatonin, an Antineurodegenerative Agent

Piyarat Govitrapong

Center for Neuroscience and Department of Pharmacology, Faculty of Science, Neuro-Behavioural Biology Center, Institute of Science and Technology for Research and Development Mahidol University, Thailand

Introduction

The neuropathology, and the etiology of different neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) share many common immunocytochemical, and structural features. Progress in neurogenetics allowed dissecting multiple causes for what once was simply known as PD, which is now split into several different disorders [1]. Despite multiple divergent causes, the pathogenesis of these diseases converges to common effector mechanism [2]. Oxidative damage has been suggested to be the primary cause of aging and age-associated neurodegenerative diseases like AD, PD and HD. Because of high rate of oxygen consumption and high content of polyunsaturated fatty acids in the brain, it exhibits increased vulnerability to oxidative stress.

Augmented free radical damage to lipids, proteins and nucleic acids has been reported for the substantia nigra (SN) of parkinsonian patients [3]. Therefore, numerous compounds with antioxidant properties have been suggested for treatment of PD and other neurodegenerative diseases [4-7]. Among these substances, melatonin is unique for several reasons. Its production decreases with the advancement of age, a fact that has been suggested to be one of the major causes of age-associated neurodegenerative diseases [8-11]. This review focuses on the role of melatonin in the etiology of neurodegenerative disorders like PD and on the therapeutic potential of melatonin in these pathologies.

Models for study Parkinson's disease

PD signs begin to appear when neuronal damage exceeds a threshold of 70-80% of dopamine (DA) nerve terminals in the striatum and 50-60% of dopaminergic neurons in SNpc. Thus, only symptomatic treatments of PD such as levodopa (L-dopa) are conventionally used [12], because it is usually too late to prevent neuronal death when the first symptoms of the disease are observed. Numerous research groups have focused their attention on animal models of PD, which would eventually allow the testing of neuroprotective strategies that would identify the key molecular steps in the neurodegenerative process [13,14]. These animal models may provide additional clues for therapeutic strategies that slow or halt the neurodegenerative process; this could have a major impact in the treatment of PD. However, to date, no drug has yet been established to have a neuroprotective effect nor has any drug been approved as a neuroprotective agent against PD [15].

In 1982, Langston and co-workers [16] first described that young heroin users developed a rapidly progressive parkinsonian-like syndrome. It was further confirmed that the drug contained a contaminant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was ultimately the chemical responsible for the PD syndrome [16]. In humans and monkeys, MPTP elicits irreversible and severe parkinsonian-like signs characterized by all the features observed in PD, i.e., tremor, rigidity, slowness of

movement, and postural instability. MPTP readily crosses the blood-brain barrier and is converted into the active toxin, 1-methyl-4-phenyl-pyridinium (MPP^+) by monoamine oxidase B (MAO-B), present in glial cells. MPP^+ is a high-affinity substrate for the dopamine transporter and can be concentrated in the synaptosomal vesicles [17]. MPP^+ also accumulates in mitochondria where it causes oxidative phosphorylation impairment by inhibiting complex I of the mitochondrial electron transport chain [18]. These findings explain the induction of specific neurodegenerative signs in the dopamineric neurons.

Other toxin-based models, frequently used to induce dopaminergic neurodegeneration, include the neurotoxin 6-hydroxydopamine (6-OHDA) and, more recently, the pesticide paraquat and the herbicide rotenone. Each of these toxins triggers the formation of ROS/RNS. The herbicide paraquat (N,N'-dimethyl-4-4'-bipiridinium), which shares a structural similarity to MPP^+ and is present in the environment, is also a toxin model of PD which causes the formation of O_2^- [20]. The herbicide rotenone was recently found to be a parkinsonism-inducing toxin. This compound, extracted form tropical plants, inhibits mitochondrial complex I at the same site as MPP+ and selectively induces nigrostriatal dopaminergic degeneration and causes the formation of fibrillar elements similar to those seen in Lewy bodies [34].

Amphetamine induces parkinsoian-like neurotoxicity

Methamphetamine (METH) is a drug of abuse and also a neurotoxin that may cause long lasting changes in the dopaminergic pathways of CNS [10]. METH treatment is known to induce nigro-striatal damage in experimental animals and also in humans [22], and therefore, it is considered as one of the models for drug-induced parkinsonism [22]. METH- induced release of DA from vesicles to cytosol and to extracellular space along with formation of free radicals is thought to one of the main mechanisms involved in METH-induced neurotoxicity [23].

Our recent study [24] suggests that METH induces dopaminergic cell line, SK-N-SH cell death by generating reactive oxygen species (ROS) and depleting the intracellular ATP levels. We observed a significant increase in ROS production in a time-dependent manner in these cells after treatment with METH. The ROS levels were significantly elevated within 30 min of METH treatment and then returned to control values within 4 h. These data suggest that the formation of ROS may be an early signaling event that mediates cell death caused by METH and correlate with previous observations reported in various neurodegenerative diseases [12]. The oxygen-based free radical theory suggests that formation of toxic radicals from DA might be the main determinant of METH-induced neurotoxicity [9]. METH has been reported to produce oxidative stress [25] via increasing hydroxyl radical in the brain [26]. Similar to other parkinsonism-inducing neurotoxins such as MPTP or 6-OHDA [1], which selectively damage dopaminergic neurons, METH-induced dopaminergic toxicity occurs in part by creating oxidative stress.

Any increase in ROS can cause mitochondrial dysfunction. It has been shown that METH, due to its lipophilicity, can diffuse through cell membranes including intracellular organelles (i.e., mitochondria), where it disrupts the electrochemical gradient. It is thus possible that METH not only kills neurons by a direct production of free radicals but also by triggering the mitochondria-mediated apoptotic cascade. In our rercent study, we observed a dose-dependent decrease in intracellular ATP

levels by METH (24). METH can disrupt the electron transport chain by inhibiting complex I activity, an event which may be associated with the decrease in ATP production. These studies clearly suggest that METH acts on mitochondria and depletes ATP levels. The consequences of mitochondrial impairment may force the cell to suffer from dysfunctional state or trigger apoptotic cell death depending on the severity of the insult [27].

Our recent study in mice [28] has shown that repeated treatment of METH induced significant reduction in striatal complex I and tyrosine hydroxylase expression, significant increase in lipid peroxidation in the striatum and cerebral cortex. Moreover the striatal CoQ_{10} is proportional to the reduction in striatal ^{18}F-DOPA uptake, detected by microPET scanner, in repeated METH-treated mice [28].

Melatonin

Melatonin is a methoxyindole secreted mainly, by the pineal gland. Once formed melatonin is not stored within the pineal gland but diffuses out into the capillary blood [29] and CSF [30]. Levels of melatonin released to the CSF were found to be 5 to 10 (up to 30) times higher than those simultaneously measured in the blood [30]. Brain tissues have higher melatonin levels than other tissues in the body [31].

Evidence accumulated since the discovery of melatonin five decades ago, indicates that this hormone reaches virtually all tissues to influence diverse aspects of biological function in species ranging from humans to unicellular organisms. Its multiple roles in mammalian physiology include the modulation of neuroendocrine function, circadian rhythmicity, immune function, and reproductive activity [32, 33]. An increasing body of evidence indicates that melatonin can exert neuroprotective effects in various models of neurodegeneration [34-36].

Neuroprotective effect of melatonin in Parkinson's disease

The age-associated decline in melatonin production and the flattening of melatonin rhythm may be major contributing factors to the increased levels of oxidative stress and associated degenerative changes seen at old age. The first evidence of a relationship between melatonin and parkinsonism came from the report of a diminished pineal activity and subsequent reduction in circulating melatonin concentration in PD patients [37]. Melatonin reduces oxidative stress and rescues dopaminergic neurons in different models of PD. Melatonin was found to inhibit the prooxidant effects of DA and L-dopa *in vitro* [38]. In addition, Khaldy et al. [39] showed that melatonin was more effective than the vitamin E analog, trolox, in preventing DA auto-oxidation. It was reported that melatonin administration prevented lipid peroxidation induced by MPTP treatment in striatum and hippocampus of mice [40]. Considerable evidence of the direct antioxidant action of melatonin has recently been reported. Jou et al. [41] showed, using confocal microscopy, that melatonin abolishes the generation of mitochondria ROS during H_2O_2-induced cell damage of rat astrocytes; this inhibition precedes cell death, which occurs hours later in cells treated with H_2O_2 only.

It was suggested that depending of its concentration, melatonin could recruit different strategies to fight against oxidative stress [42]. It is well known that melatonin effects on antioxidant enzymes are already seen at physiological

concentrations and direct scavenging has been demonstrated at pharmacological concentration [43]. Our recent results indicate that pretreatment with melatonin markedly prevented the loss of cell viability caused by D-amphetamine (AMPH) treatment. It prevented the overproduction of ROS, lipid peroxidation, depletion of intracellular ATP levels and induction of α-synuclein expression, caused by AMPH [44].

Our recent results have shown that pretreatment with melatonin prevents the ATP depletion caused by AMPH treatment in the SK-N-SH cells. Melatonin was found to increase mitochondrial respiration and ATP synthesis, in conjunction with rises in complex I and IV activities [45]. *In vitro* and *in vivo* experiments have shown that melatonin can influence mitochondrial homeostasis. It was suggested that the actions of melatonin on mitochondria might be mediated via at least three mechanisms. First, antioxidant and free radical scavenging properties of the indoleamine protect the organelle from oxidative damage. Secondly, its actions at the mitochondrial DNA level increase the expression of complex IV. Thirdly, a direct action interaction of melatonin with the mitochondrial transition pore was found recently [46].

Most previous studies reported the general neuroprotective effect of melatonin under a variety of challenges, for example: 6-OHDA [47-50], MPP^+ [55] rotenone [56]. Some of the molecular pathways involved in melatonin's neuroprotective actions have been elucidated. The antioxidant action of melatonin abolish NF-κB activation [57] and c-Jun phosphorylation [58], events that usually precede 6-OHDA induced apoptotic cell death. However, the protective effect of melatonin on the induction of α-synuclein expression has not been investigated. Our recent data have shown that melatonin significantly decreases the expression of α-synuclein induced by AMPH in SK-N-SH cells [44].

Our recent study demonstrated that AMPH treatment caused disturbance in DA system that may predispose individuals to Parkinsonism. Furthermore, AMPH neurotoxicity offers a new scenario for the biochemical pharmacology aimed at interfering with these steps which might be tagged by selective therapeutic agents. We demonstrated that melatonin provides neuroprotection against AMPH-induced neurotoxicity in the SK-N-SH cells. These findings may have implications for the elucidation of cellular mechanisms of AMPH and, by extension, in neurodegeneration diseases such as PD, which involves pathology in the nigrostriatal DA pathway. The use of melatonin could be explored as a therapeutic approach in various neurodegenerative disorders such as AD, PD and HD.

Acknowledgements

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S1-2 Novel Therapeutic Strategies for Neurodegenerative Diseases

Chuthamanee Suthisisang, BPharm, Ph.D.
Department of Pharmacology, Faculty of Pharmacy, Mahidol University

Novel Therapeutic Strategies for Neurodegenerative Diseases

Chuthamanee Suthisisang, BPharm,Ph.D.
Department of Pharmacology
Faculty of Pharmacy
Mahidol University

Example of neurodegenerative disorders with major neurotransmitter deficit

- Alzheimer's disease
- Dementia with Lewy body
- Parkinson's disease
- Amyotrophic lateral sclerosis

Features of neurodegenerative disorders characterized by aggregation and deposition of abnormal protein

Science 2002;296:1991-5

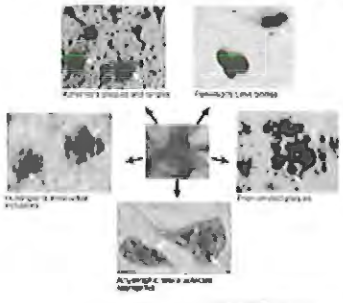
Protein folding

- In order to be functionally active, a protein has to acquire a unique 3D conformation via a complicated folding pathway, which is described by the primary amino acid sequence and the local cellular environment.
- Protein folding is vital for a living organism because it adds flesh to the gene skeleton.
- Within the cellular environment, which is highly viscous, many proteins cannot fold properly by themselves and require the assistance of a special kind of ubiquitous protein, the molecular ***chaperones***.

Protein misfolding

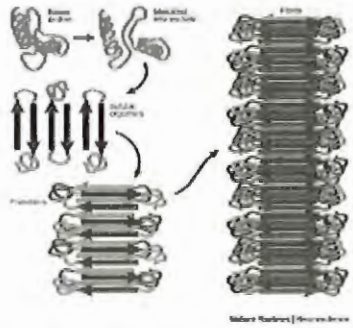
- Proteins that are not able to achieve the native state, due either to an unwanted mutation in their amino acid sequence or simply because of an error in the folding process, are recognized as ***misfolded*** and subsequently targeted to a degradation pathway.

Cerebral Aggregates In Neurodegenerative diseases

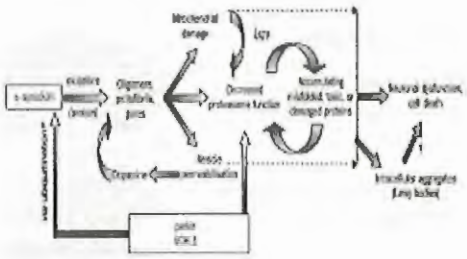


Nature Rev Neuroscience 2003;4:49-60

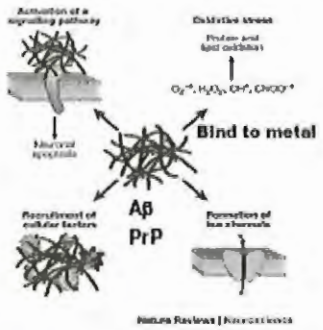
Pathway leading to protein misfolding and aggregation



Proposed pathogenesis of Parkinson's disease



Model for the neurotoxic mechanisms of misfolded aggregates



Neurodegenerative diseases in which metal interactions might mediate protein aggregation

- Aβ – Cu, Fe, Zn
- PrP – Cu
- SOD – Cu
- α-Synuclein - Cu, Fe

The role of metals in the process of amyloid beta peptide polymerisation

Both Cu^{++} and Zn^{++} binding to Aß is supposed to have a structural role

⇓

Both metal ions strongly promote aggregation by creating links between one peptide and next one in the nascent fibril

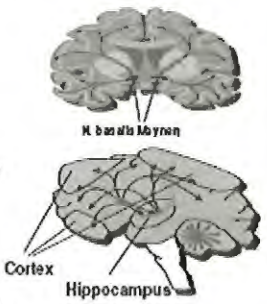
Cu^{++} undergoes mainly	intrapeptide binding ⇒ inducing *conformational changes*
Zn^{++} promotes	interpeptide binding ⇒ favouring *aggregation*

Aβ protofibril toxicity

- Activate reactive oxygen species
- Activate stress kinase signalling
- Activate GSK3β (valproate inhibit GSK3β)
- Activate tau phosphorylation
- Activate apopototic cascade

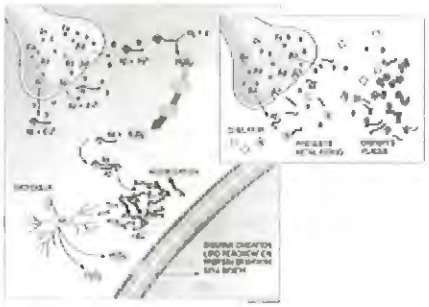
The cholinergic deficit in AD underlies the clinical symptomatology

- Cholinergic deficit
  - progressive loss of cholinergic neurones
  - progressive decrease in available ACh
  - impairment in ADL, behaviour and cognition



Bartus et al., 1982; Cummings and Back, 1998; Perry et al., 1978

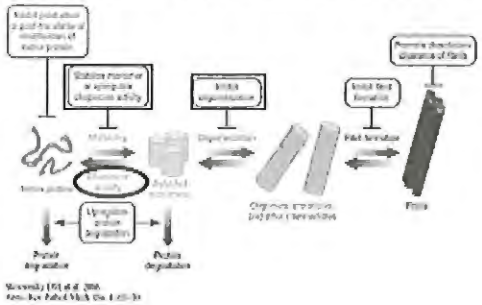
Chelation of heavy metals reduces Aβ oligomerization and levels of oxidative stress



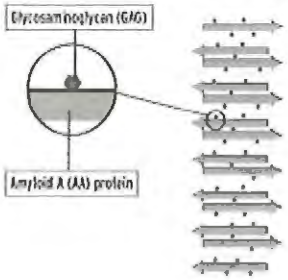
Aβ/Cu in the presence of H_2O_2 will generate Aβ oligomers (crosslinks)

Michaels 2003

Targeting protein misfolding in neurodegenerative diseases

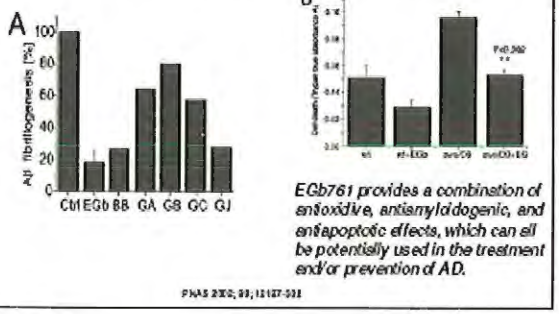


Aβ monomer stabilizer



- GAG mimetic
- Clioquinol
- Polyphenol
- Tramiprosate
- Bilobalides

Inhibition of amyloid-β aggregation and caspase-3 activation by the *Ginkgo biloba* extract EGb761



EGb761 provides a combination of antioxidive, antiamyloidogenic, and antiapoptotic effects, which can all be potentially used in the treatment and/or prevention of AD.

PNAS 2002; 99; 13187-331

Ginkgo biloba and Prevention of Dementia: the GEM Study

(PI: Steven DeKosky, MD)

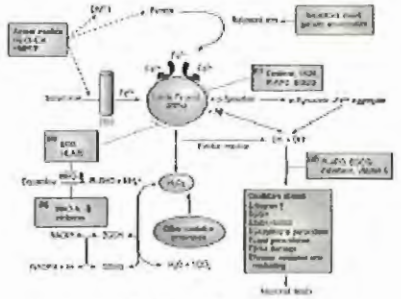
Ginkgo Evaluation of Memory Study

Four major aims:

- Will Ginkgo slow development of dementia?
- Will it reduce rates of cognitive loss in aging?
- Will it reduce vascular disease symptoms?
- Will it decrease total mortality?

Funded by the National Institutes of Health:
- National Center for Complementary and Alternative Medicine (NCCAM)
- National Institute on Aging
- National Heart, Lung, and Blood Institute

Iron chelation as a target in neurodegenerative diseases



TiPs 1005;26: 27-35

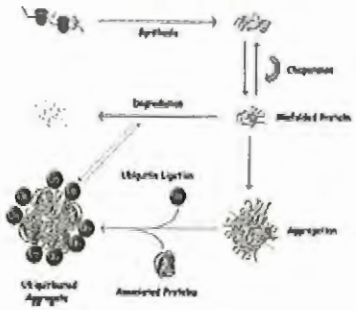
Chaparone proteins

- Molecular chaperones assist other proteins to achieve a functionally active 3D structure and thus prevent the formation of a misfolded or aggregated structure.
- Enhancing folding efficiency by influencing the kinetics of the process and inhibiting events that lead to unproductive end points (e.g. aggregation).
- Chaperones are able to distinguish between the native and non-native states of targeted proteins.
- *Increased chaperone expression* can suppress the neurotoxicity caused by protein misfolding.

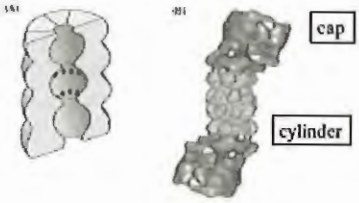
Molecular chaperones

- several distinct classes of sequence-conserved proteins, most of which are stress inducible like heat shock proteins (Hsp)
- Hsp100, Hsp90, Hsp70, Hsp60 and the small HSPS

Protein turnover in neuronal diseases



The proteasome degrades unwanted proteins

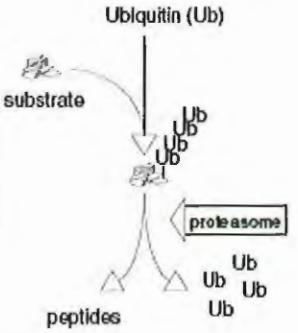


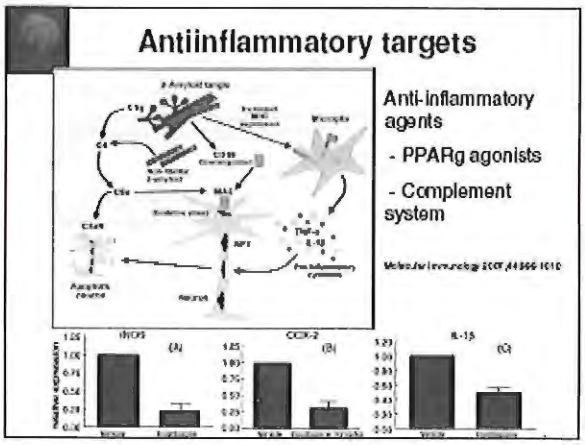
Proteasomes contain a central cylinder formed of proteases whose active sites face inward.

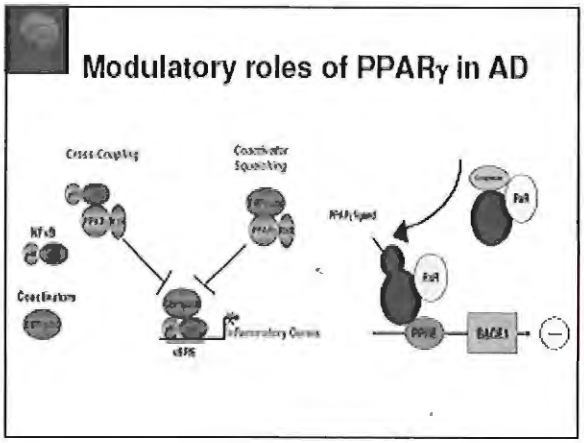
Attachment of ubiquitin regulates a protein being degraded by the proteasome

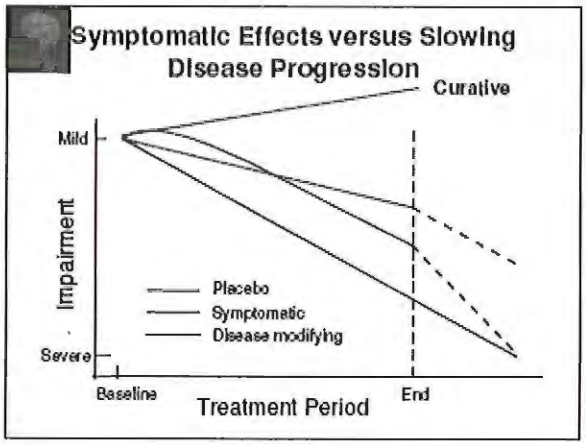
Ubiquitin is a 76 amino acid protein that if gated onto a protein substrate and allows that substrate to be recognized by the proteasome. Ub is recycled and the substrate is degraded.

~80% of all cellular proteins are degraded by the proteasome.




Antiinflammatory targets
Anti-inflammatory agents
- PPARg agonists
- Complement system


Modulatory roles of PPARγ in AD


Symptomatic Effects versus Slowing Disease Progression
Curative
Mild
Impairment
Severe
Placebo
Symptomatic
Disease modifying
Baseline
End
Treatment Period

S2-1 Roles of Device Therapy and Fish Oil in the Prevention of Arrhythmic Death

รองศาสตราจารย์ ดร. นพ. นิพนธ์ ฉัตรทิพากร

ศูนย์วิจัยและฝึกอบรมสาขาโรคทางไฟฟ้าของหัวใจ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

Sudden cardiac death has been a major cause of death in most industrialized nations around the world.[1] It is known that ventricular fibrillation (VF) is mainly responsible for this fatality.[2] Currently, the only effective clinical treatment of VF is to give a strong electrical energy to a fibrillating heart, and is known as electrical defibrillation.[3] An external defibrillator has been used as a device to defibrillate VF in patients for many decades. However, this currently known automated external defibrillator (AED) still has significant limitations. When VF attacks one victim, at least three crucial factors are needed. First, there must be an AED available in that location where the victim collapses. Second, there must be at least a person to present nearby the victim. And lastly, that person must know how to use the AED to defibrillate the victim. Due to these limitations, a number of VF victims could not be saved and the mortality rate had been very high. With the introduction of an implantable cardioverter defibrillator (ICD), the mortality rate has been significantly decreased and a number of lives have been saved.[1]

An ICD is a device implanted into patients with risk of developing VF. This device can detect VF occurring in that person implanted with an ICD and automatically deliver a shock to defibrillate the heart within tens of seconds. Clinical trials on ICD have been done and demonstrated that ICDs significantly decreased mortality rates compared to standard pharmacological therapy.[4] Currently, ICD is well accepted as a gold standard treatment for sudden cardiac death. Despite its clinical benefits, ICDs is still far from ideal in treating VF. Most VF victims already have sick hearts. Since successful defibrillation requires the delivery of high-energy shock to the heart, this may cause undesired effects in the short and long run of those sick-heart patients. Therefore, attempts to decrease the shock strength required to defibrillate have been under investigation.[5-7] To achieve this goal, understanding how the shock terminates VF is crucial.

Pharmacological interventions have been another approach in minimizing the shock energy required to successfully defibrillate.[8, 9] Many anti-arrhythmic drugs have been demonstrated to have harmful effects as well as increase energy requirement for defibrillation,[10] Natural products as well as herbal medicine have also been investigated for their possible beneficial effects on arrhythmia prevention and defibrillation.[11]

In the past decades, fish oil, a product enriched in deep sea fish, which is known for its cardioprotective effect has been extensively investigated.[12-16] However, its role in defibrillation has never been investigated. Recent studies have demonstrated that intravenous administration of docosahexaenoic acid could significantly raise the arrhythmia induction threshold as well as reduce the arrhythmia induction window, suggesting that it has potential benefits in the prevention of arrhythmias including ventricular fibrillation.[7] The effect of fish oil on defibrillation has been investigated in only a few studies, and the results demonstrated no reduction in defibrillation threshold after fish oil administration. Several limitations, however, are applied to those reports including that only one concentration of fish oil was used in those studies.[17, 18] Future studies are needed to warranty the definite effects of fish oil on defibrillation efficacy.

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S2-2 Myocardial ischemia reperfusion injury

Assist. Dr. Payong Wanikiat
Department of Pharmacology, Faculty of Science, Mahidol University

Myocardial infarction is the major cause of death in the world. Over the last two decades, coronary reperfusion therapy has become established for the management of acute myocardial infarction (AMI). Reperfusion therapy encompasses both thrombolytic therapy and percutaneous coronary intervention (PCI). However, restoration of blood flow to previously ischemic myocardium results in the so-called ischemia/reperfusion (IR)-injury.

The different clinical manifestations of this injury include reperfusion arrhythmia, myocyte death, myocardial stunning and endothelial- and microvascular dysfunction including the no-reflow phenomenon. The pathogenesis of IR-injury consists of many mechanisms.

In humans, the most common reperfusion arrhythmia is an accelerated idioventricular rhythm. However, ventricular tachycardia and ventricular fibrillation remain the most important causes of sudden death following spontaneous restoration of antegrade flow. It has been suggested that oxygen-derived free radicals might play a key role in the genesis of ventricular arrhythmias.

Myocardial stunning is defined as "prolonged postischemic mechanical dysfunction that persists after reperfusion, of previously ischemic tissue in the absence of irreversible damage, including myocardial necrosis. Stunning is an important causative factor in the development of ischemic cardiomyopathy, wherein repeated episodes of myocardial ischemia and reperfusion may lead to the development of heart failure.

Cardiomyocyte cell death during ischemia is named "oncosis", during reperfusion it is a part of the reperfusion injury. Development of cardiomyocyte contracture seems to be the primary cause for necrotic cardiomyocyte injury during the earliest phase of reperfusion. Thereafter, various additional causes can lead to further increment of cell death either by necrosis or apoptosis.

A combination of endothelial dysfunction, microvascular obstruction, edema and oxidative stress, is responsible for the pathogenesis of microvascular dysfunction after IR. Severe microvascular dysfunction may limit adequate perfusion after reperfusion, a phenomenon termed "no reflow". This phenomenon is characterized by decreased resting myocardial blood flow, ultrastructural vascular alterations, and distinct areas of hypoperfusion. It is associated with an increasd incidence of AMI, myocardial rupture, and death.

The underlying pathophysiological mechanisms of IR have not been fully elucidated. It has been suggested that an overproduction of oxygen-derived free radicals and intracellular calcium overload or redistribution during the first minute of reflow might be involved. However, oxygen-derived free radicals and hypercontracture due to calcium-overload are not the only cancidates responsible for reperfusion injury. Other factors of importance in the pathogenesis of reperfusion injury include platelet-and neutrophil-mediated injury, the rennin-angiotensin system and the complement activation.

There are two possibilities to influence the injury caused by ischemia: Induction of preconditioning and a pharmacological approach. Preconditioning can be induced by ischemia itself, and by drugs. These drugs are adenosine, opioids, bradykinin B_2-receptor antagonists, ACE-inhibitors and glibenclamide. The pharmacological approach for preventing ischemia induced injury contains two strategies: L-arginine supplementation and Na^+/H^+ -exchange inhibitors. Reperfusion injury can be treated by NO donors, repolarizing solutions, adenosine-receptor agonists, Na^+/H^+ -exchange inhibitors, magnesium and platelet inhibitors.

P01 Thiopurine S-methyltransferase Polymorphism Correlated with Azathioprine-Induced Hematotoxicity in Kidney Transplant Recipients

Susothorn Angsuthum[1], Wichittra Tassaneeyakul[1], Cholatip Pongskul[2], Dhavee Sirivongs[2], Wongwiwat Tassaneeyakul[3], Yingyos Avihingsanon[4], SudaVannaprasaht[1]

[1]Department of Pharmacology and [2]Department of Medicine, Faculty of Medicine, Khon Kaen University. [3]Department of Toxicology, Faculty of Pharmaceutical Science, Khon Kaen University. [4]Department of Medicine, Faculty of Medicine, Chulalongkorn University.
E-mail: susotorn_jao@hotmail.com

Abstract

Introduction: Thiopurine S-methyltransferase (TPMT) is a key enzyme responsible for the inactivation of thiopurine drugs such as azathioprine (AZA), an effective immunosuppressant. TPMT is polymorphic in man. Patients with non-functional TPMT mutant alleles exhibit a reduction in TPMT activity and are at higher risk for developing fatal hematotoxicity although a standard dose of thiopurines is given. TPMT*3C (A719G) is the most prevalence of non-functional mutant alleles found in Thai population with the prevalence of 9.5%.
Objective: To investigate the correlation between TPMT genetic polymorphism and AZA-induced hematotoxicity in kidney transplant (KT) recipients.
Materials and Methods: Medical record and clinical data of the KT recipients were retrospectively evaluated during AZA therapy. TPMT*3C was identified by real-time PCR and the TPMT activity in RBC was measured using HPLC technique.
Results: Eight out of 130 KT recipients (6.15%; 95% CI 2.69 – 11.77%) were identified as heterozygous TPMT*1/*3C and exhibited significant reduction in TPMT activity comparing with those of the wild type group (17.67 ± 11.92 vs. 38.78 ± 10.59 nmol 6-MTG. g^{-1}Hb. hr^{-1}, $p < 0.001$). Our results showed that the heterozygous of *TPMT*1/*3C* was at 25.24 folds (90% CI 2.23 – 285.29, $p < 0.01$) higher risk for AZA-induced hematotoxicity after receiving a standard dose of AZA.
Conclusion: The presence of polymorphic TPMT*3C in the KT recipients was correlated well with reduction of TPMT activity in RBC and AZA-induced hematotoxicity. Determination of TPMT status may be useful for selection and dosage adjustment of AZA in KT recipients to ensure safety of the drug.
Keywords: genetic polymorphism, thiopurine S-methyltransferase, azathioprine, hematotoxicity, kidney transplantation

Introduction

Azathioprine (AZA)is a thiopurine drug used as an effective immunosuppressant in organ-transplantation recipients. AZA undergoes enzymatic inactivation by xanthine oxidase (XO) and thiopurine-S methyltransferase (TPMT). Because of the absence of XO enzyme in hematopoietic tissue, TPMT appears to be the key enzyme responsible for inactivation of AZA in these tissues. *TPMT* genetic polymorphism is an important factor responsible for large inter and intra-ethnic differences of TPMT enzyme activity including individual differences in thiopurine toxicity and therapeutic efficacy. Patients with low or intermediate TPMT activity are at high risk for excessive active metabolite accumulation in the hematopoietic tissue

and the development of fatal hematotoxicity after receiving a standard dose of thiopurines (1). TPMT*3C is the most prevalence non-functional mutant allele found in Asian population (1). The prevalence of TPMT*3C mutation in northeastern Thai healthy volunteers was 9.5% (2). Moreover, the frequency of this allele in Thai northeastern population is significant higher than other Asian population (2).

Therefore, the aim of this study was to investigate the clinical significant correlation between TPMT genetic polymorphism and the development of AZA-induced hematotoxicity in the KT recipients to provide a perspective on TPMT status estimation in routine clinical practice before prescribing AZA in the KT recipients.

Methods

One hundred and thirty KT recipients who received AZA as a part of standard immunosuppressive regimen were recruited from Srinagarind Hospital, Khon Kaen University, Khon Kaen and Chulalongkorn Hospital, Chulalongkorn University, Bangkok. The protocol of this study was approved by the Ethical Committee on Human Research, Khon Kaen University and Chulalongkorn University, Thailand.

Peripheral venous blood samples were obtained for TPMT genotyping analysis and the TPMT enzyme activity was measured in every routine clinic visits.

For TPMT genotyping analysis, genomic DNA was isolated from total peripheral WBC by QIAGEN blood extraction kit and the presence of TPMT*3C mutant allele (A719G) was identified by our developed real-time PCR method using Hydrolysis (Taqman®) probes.

For TPMT activity measurement, RBC were separated and lysed with 0.02 mM phosphate buffer pH 7.4. TPMT activity was measured in RBC lysate using 6-thioguanine (6-TG) as substrate according to Ford LT and Berg JD method (3) with minor modifications. 6-Methylthioguanine (6-MTG), the highly fluorescent metabolite was measured using HPLC technique. The specific activity of TPMT enzyme was expressed as nmol 6-MTG. g^{-1}Hb. hr^{-1}.

For the investigation of AZA-induced hematotoxicity, individual hematological laboratory and clinical information were obtained from hospital inpatient and outpatient case history charts and collected for 6 months after receiving AZA therapy. The presence of leukopenia and/or neutropenia and/or thrombocytopenia and/or AZA dose reduction-discontinuation was used as inclusion criteria for AZA-induced hematotoxicity in this study. Leukopenia was defined as white blood cell (WBC) count < 3,000 cells/cu.mm^3, neutropenia was defined as % absolute neutrophil count (%ANC) < 500 cells/cu.mm^3 and thrombocytopenia was defined as platelet count < 100,000 cells/cu.mm^3.

The incidence of *TPMT* genetic polymorphism in 130 KT recipients was expressed as percent (%) genotype frequency. Mean TPMT activity and AZA dose were compared using t-test. The correlation between *TPMT* genetic polymorphism and AZA-induced hematotoxicity were determined using Fisher's exact test and Odd ratio (OR test). Statistical analysis was performed using STATA® 8.0 computer software. The p-values less than 0.05 were considered statistically significance.

Results

The TPMT genotyping analyses revealed that 122 out of 130 KT recipients (93.85%; 95% CI 88.23 – 97.31%) were homozygous of TPMT*1. Whereas, 8 out of 130 KT recipients (6.15%; 95% CI 2.69 – 11.77%) were heterozygous of TPMT*1/*3C. However, homozygous of TPMT*3C was not detected among these 130 KT recipients (Table 1).

The inter-individual difference TPMT enzyme activity in RBC was observed in this study. TPMT activity measured in 112 KT recipients showed 44-fold variability, ranging from 2 up to 88 nmol 6-MTG/gHb^{-1}/hr^{-1}. TPMT genotype was correlated well with TPMT activity in which the TPMT*1/*3C heterozygous group exhibited significant TPMT activity reduction compared with that of the wild type group which exhibited high TPMT activity (17.67 ± 11.92 nmol vs. 38.78 ± 10.59 nmol 6-MTG.g^{-1}Hb.hr^{-1}, $p < 0.001$) (Table 1).

From the review of patients hematological laboratory and clinical information, a significant higher incidence of leucopenia and thrombocytopenia during 6 months of AZA treatment were observed in the heterozygous of TPMT*1/*3C group ($p < 0.05$) (Table 2). Moreover, the odd ratio indicated that the heterozygous of TPMT*1/*3C are at 25.24 folds (90% CI 2.23 – 285.29, $p < 0.01$) higher risk for the development of hematotoxicity than that of the wild type group when commencing on standard dose of AZA (Table 3).

Considering the AZA dose, the heterozygous and the wild type groups received similar doses of AZA (mg/kg/day) at the initiation of the therapy (1.29 ± 0.44 vs. 1.39 ± 0.49, respectively, $p > 0.05$). The incidence of AZA dose-reduction or discontinuation was higher in the heterozygous group but the difference was not statistically significant. However, 2 of 8 (25%) heterozygous of TPMT*1/*3C KT recipients required reduction of AZA dose administration during 6 months of AZA therapy whereas 4 of 8 (50%) heterozygous of TPMT*1/*3C had AZA discontinued. Our results suggest that most of the KT recipients with TPMT*3C mutant allele could not tolerate with a standard dose of AZA therapy and required the dosage adjustment or discontinuation of AZA.

Table 1: TPMT genotype frequency (%) in 130 KT recipients and mean (range) values of TPMT activity based on their genotype.

***TPMT* genotype**	**N**	**Frequency (%)** (95% CI)	**Mean (Range) TPMT activity** (nmol 6-MTG.g^{-1}Hb.hr^{-1})
Homozygous *1/*1	122	93.85 % (88.23 – 97.31)	38.78 ± 10.59 (19.83 – 88.85)
Heterozygous *1/*3C	8	6.15 % (2.69 – 11.77)	17.67 ± 11.92* (1.73 - 26.82)
Total	130	100 %	38.03 ± 11.29 (1.73 – 88.85)

*Significant compared with *TPMT*1/*1* group ($p < 0.001$).

Table 2: Incidence of hematotoxicity and AZA dose reduction or discontinuation between wild type and heterozygous of *TPMT*1/*3C* group.

Characteristics	***1/*1* (n = 122)**	***1/*3C* (n = 8)**	**p-value**
AZA-induced hematotoxicity	49 (40.16%)	8 (100%)*	0.001
Leukopenia (WBC < 3,000 cells/cu.mm^3)	12 (9.84%)	3 (37.5%)*	0.049
Thrombocytopenia (platelet < 100,000 cells/cu.mm^3)	18 (14.75%)	5 (62.5%)*	0.004
Neutropenia (%ANC < 500 cells/cu.mm^3)	1 (0.82%)	0 (0%)	-
AZA dose-reduction or discontinuation	45 (36.89%)	6 (75%)	0.056

*Significant compared with *TPMT*1/*1* group ($p < 0.05$).

Table 3: AZA-induced hematotoxicity in KT-recipients segregated by TPMT genotype.

AZA-received KT recipients	*TPMT* genotype		Total
	**1/*3C*	**1/*1*	
hematotoxicity	8	49	57
Non-hematotoxicity	0	73	73
Total	8	122	130
Odd ratio = 25.24, 90%CI = 2.23 – 285.29, $P < 0.01$			

Discussion

Our retrospective study showed a significant statistical and clinical correlation between the development of AZA-induced hematotoxicity and the presence of TPMT*3C mutant allele in the KT recipients. In this study, the risk for development of hematotoxicity in KT recipients with TPMT*3C is 25.24 folds (90% CI 2.23–285.29, $p < 0.01$) higher than that of the wild type group.

The incidence of leukopenia and thrombocytopenia was significantly higher in these heterozygous TPMT*1/*3C. The presence of anemia was not used as an inclusion criteria for AZA-induced hematotoxicity in this study because anemia is a very common phenomenon after kidney transplantation and may be the result of not only AZA administration but may include allograft dysfunction, blood lost and nutritional-induced anemia.

The overall frequency of TPMT*3C mutant allele in this study was 6.02% which was lower than that reported previously in Thai northeastern healthy subject (2). This may be a result from the highly-selected study population. Moreover, the inter-ethic TPMT genetic variability should be considered and may be used to explain this lower incidence because most KT recipients in this study were recruited from the center of Thailand. Moreover, the mean TPMT activity in RBC of the wild type group in this study was rather lower than that of the previous report (3) This also may indicate the inter-ethic variation of TPMT activity.

Kidney transplantation is the best treatment strategy for end-stage renal disease. However, it is a high-cost treatment which covers many expenses associated with kidney transplants, including medications. After transplantation, the patients need to take continuous lifelong immunosuppressive agents to prevent kidney graft-rejection. AZA remains a cornerstone of immunosuppressive therapy, despite the development of newer agents. AZA is a low price medication and is not nephrotoxic. The prospective identification of individuals TPMT activity and/or TPMT genotype would be useful to optimize individualize dosage regimen of AZA to minimize risk of hematotoxicity.

Conclusion

In this study, the presence of TPMT*3C in kidney transplant recipients was correlated well with significant reduction of TPMT enzyme activity and the development of AZA-induced hematotoxicity. Therefore, TPMT status estimation by mutation analysis of TPMT non-functional mutant allele and measurement of TPMT enzyme activity in RBC may be useful as markers for identifying at-risk patients to optimize the dosage regimen for obtaining optimal therapeutic efficacy and minimizing hematotoxicity before prescribing AZA.

Acknowledgements

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P02 Inhibitory Effect and Mechanism-Based Inhibition of Thai Herbal Plants on CYP3A4 and CYP2D6 Activities

Weeraya Dumrongsakunchai[1,2], Vichien Attakornvattana[3], Aimon Somanabandhu[4], Suda Vannaprasaht[2], Wongwiwat Tassaneeyakul[5], Wichittra Tassaneeyakul[2]

[1]*Graduated student, Graduate School, Khon Kaen University.*
[2]*Department of Pharmacology, Faculty of Medicine, Khon Kaen University.*
[3]*Queen Sirikit Hospital, Sattahip, Chonburi.*
[4]*Department of Pharmacoglogy, Faculty of Pharmacy, Mahidol University.*
[5]*Department of Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen, University.*
E-mail : Weeraya_44@hotmail.com

Abstract

Introduction: Herbal plants becomes a popular form of healthcare in Thailand. Due to the fact that herbal medicines users tend to have chronic conditions and may receive prescribed drugs concomitantly raises the potential of herb-drug interactions. Safety issues associated with these herbal medicines are mostly unknown and remained under-researched. Several herbal medicines have recently been reported to interact with prescribed drugs either by inhibiting or inducing the activity of cytochromes P450 (CYP).

Objective: To investigate the inhibitory effect of commonly used Thai herbal plants on CYP3A4 and CYP2D6, the major drug metabolizing enzymes in human and involved in the metabolism of several clinically used drugs.

Materials and methods: The inhibitory effects and mechanism-based inhibition of 18 commonly used Thai herbs on the activity of CYP3A4 and CYP2D6 were determined in vitro using human microsomal testosterone 6β-hydroxylase and dextromethorphan O-demethylase as markers of CYP3A4 and CYP2D6 activity, respectively.

Results: The results revealed that some herbal extracts particularly the ethanolic extracts were potent inhibitors of CYP3A4 and CYP2D6. Mechanism-based inhibition on CYP3A4 activity was observed for the ethanolic and aqueous extracts of some herbs.

Conclusion: The results obtained from the present study suggest the possibility of potential herb and drug interaction of commonly used Thai herbal plants. Therefore, health-care practitioners and patients should be awared when using these herbs with some prescribed drugs.

Keywords: Herb-Drug interaction, CYP3A4, CYP2D6, Thai herbal plants, Mechanism-based inhibition

Introduction

There is an increasing use of herbs in combination with therapeutic drugs. This may result in both pharmacokinetic and pharmacodynamic herb–drug interactions. Cytochromes P450 (CYP) are the main enzymes responsible for the metabolism of clinically used drugs. Of these CYP, CYP3A4, the major CYP isoform in human liver, metabolizes more than 50% of clinically used drugs whereas about 30% of these drugs are catalyzed by CYP2D6. Several lines of evidence indicate that interaction between herbs and drugs can occur via inhibition and induction of CYP. Recent *in vitro* and *in vivo* studies have demonstrated that St John's wort is a potent inducer of CYP3A4 and 2B6 (1). Moreover, St John's wort extract also inhibits the

activities of CYP1A2, CYP2C9, CYP2C19 and CYP2D6 (2). Many organosulfur compounds from garlic are competitive inhibitors of CYP2E1 (3). Many Chinese, Japanese and Indonesian herbs have been reported as the inhibitors of several CYP isoforms (4, 5). Although herbal plants have been used from ancient times in Thailand, data concerning the interaction between of Thai herbs and prescription drugs are still very limited. Thus, the aim of the present study was to determine the inhibitory effect of commonly used Thai herbal plants on human CYP3A4 and CYP2D6 activity as well as determined their mechanism of inhibition.

Methods

Preparation of herbal extract

Several commonly used Thai herbal plants were selected and extracted with aqueous or ethanol. After extraction, ethanol and water were evaporated under low pressure at 50-60 °C and freeze dry, respectively.

Inhibitory effect of Thai herbal extracts on CYP2D6 and CYP3A4

Human hepatic dextromethorphan O-demethylase and testosterone 6β-hydroxylase were used as selective markers of CYP2D6 and CYP3A4 activities, respectively (6,7). To ensure the selectivity of these markers, the concentrations of substrate around apparent *Km* values were employed (i.e. 20 μM for dextromethorphan; 200 μM for testosterone). An equal volume of water or methanol was also added to controls. In general, an incubation mixture containing aqueous or ethanolic extract of herbal plant, a substrate, human liver microsomes in 0.1 M phosphate buffer (pH 7.4) was preincubated at 37°C for 3 min. The reaction was then initiated by the addition of 1mM β-NADPH and continued at 37°C for specific periods in a shaking water bath. β-NADPH was omitted from blanks and replaced by an equal volume of phosphate buffer.

Mechanism-based inhibition of Thai herbal extracts on CYP2D6 and CYP3A4

For mechanism-based inhibition, concentration of herbal extract at around the IC_{50} values were added to an incubation mixture containing human liver microsomes and 100 mM phosphate buffer, pH 7.4. Incubation mixtures were prewarmed at 37^{0}C for 5 min and then the reaction was started by the addition of 1 mM β-NADPH and incubated for 10 min. After that the substrate was added. The reaction was terminated after being incubated for 30 or 45 min for CYP3A4 and CYP2D6 activity, respectively.

Analysis of results

IC_{50} values (concentrations of inhibitor causing 50% reduction in activity relative to the control) were calculated by linear regression analysis of the log inhibitor concentration versus percentage control activity plots. Data were presented as mean ± S.D. and analyzed by ANOVA and paired t-test. The p-values < 0.05 were considered statistical significance.

Results

Inhibitory effect of Thai herbal plants on human CYP3A4 activity

The aqueous and ethanolic extracts of some herbs (A. paniculata and C. longa) contained chemicals that can interfere with the peak of 6β-hydroxytestersterone, the metabolite of testosterone, therefore we could not determine the inhibitory effect of these extracts on human CYP3A4 activity. Dose-dependent inhibitions on CYP3A4 activity were observed for the aqueous and ethanolic extracts of herbal plants. However, the inhibitory potency of these extract was varied ranging from 0.77 to 58

μg/ml incubation (Table 1).Comparing to the known CYP3A inhibitors, the inhibitory potency of these herbs on CYP3A was less than ketoconazole but most of them were more potent than erythromycin and clarithromycin.

Inhibitory effect of Thai herbal plants on human CYP2D6 activity

Similar to the effect on human CYP3A4 activity, the aqueous and ethanolic extracts showed dose-dependent inhibition on CYP2D6 activity. However, the inhibitory potency of these extracts was varied ranging from 23 to more than 1000 μg/ml incubation (Table 1). It should be noted that some herbs (A. paniculata, C. alata, C. longa, M. hortensis, R. nasutus(root),(leaves), T. laurifolia and *Z.* officinale contained chemicals that can interfere with the peak of dextrophan, the metabolite of dextromethorphan, therefore, the inhibitory effect of these extracts on human CYP2D6 activity could not be determined. Comparing to known inhibitors of CYP2D6, the aqueous and ethanolic extracts of these herbs were less potent than quinidine, fluoxetine and paroxetine.

Table 1: IC_{50} values of ethanolic and aqueous extracts of Thai herbal plants on human CYP3A4 and CYP2D6 activities.

	IC_{50} values (μg/ml incubation) for			
	CYP3A4		*CYP2D6*	
	Ethanolic extract	*Aqueous extract*	*Ethanolic extract*	*Aqueous extract*
Known CYP3A4 inhibitor				
Ketoconazole	0.11 ± 0.08			
Erythromycin	83.33 ± 61.10			
Clarithromycin	730 ± 233.02			
Known CYP2D6 inhibitor				
Quinidine			0.97 ± 0.06	
Fluoxetine			0.04 ± 0.01	
Paroxetine			0.02 ± 0.01	
A. paniculata (ฟ้าทะลายโจร)	ND	566.7 ± 125.0	82.7 ± 6.4	ND
C. alata (ชุมเห็ดเทศ)	24.3 ± 14.3	253.33 ± 40.4	33.00 ± 25.6	ND
C. asiatica (ใบบัวบกต้นขาว)	58.3 ± 23.1	> 1,000	213.3 ± 205.0	> 1,000
C. asiatica (ใบบัวบกต้นแดง)	ND	> 1,000	83.3 ± 20.8	> 1,000
C. longa (ขมิ้นชัน)(หัวใหญ่)	ND	76.7 ± 15.3	ND	163.3 ± 41.6
C. longa (ขมิ้นชัน)(หัวเล็ก)	ND	ND	ND	430.0 ± 43.6
Cyanobacterium (สาหร่าย spirulina)	ND	> 1,000	ND	> 1,000
G. pseudochina (ว่านมหากาฬ)	136.7 ± 70.2	> 1,000	50 ± 25	563.3 ± 309.9
K. parviflora (กระชายดำ)	28 ± 19.5	120 ± 20.0	77 ± 9.54	726.67 ± 40.4
M. alba (หม่อน)	626.7 ± 58.6	> 1,000	630 ± 55.2	> 1,000
M. citrifolia (น้ำลูกยอ)	ND	> 1,000	ND	> 1,000
M. hortensis (บีบ)	13 ± 3.6	55.3 ± 14.5	33.33 ± 8.1	ND
O. aristatus (หญ้าหนวดแมว)	40 ± 8.7	286.7 ± 65.1	31.0 ± 19.5	406.7 ± 141.9
P. amarus (หญ้าลูกใต้ใบ)	0.77 ± 0.1	25.33 ± 4.6	23 ± 26.9	133.33 ± 66.6
R. nasutus.(ทองพันชั่ง)(ใบ)	> 1,000	> 1,000	ND	> 1,000
R. nasutus (ทองพันชั่ง)(ราก)	53.3 ± 11.6	853.33 ± 98.7	47.00 ± 10.8	ND
S. leucantha (หนุมานประสานกาย)	756.7 ± 204.0	ND	ND	410.0 ± 151.3
T. laurifolia (รางจืด)	203.3 ± 115.9	247.67 ± 66.6	45.00 ± 5.0	ND
Z. officinale *(ขิง)*	30.3 ± 15.1	270 ± 79.4	ND	ND

ND = not detected. Data represents mean ± S.D. form three experiments.

Mechanism-based inhibition of CYP3A4 and CYP2D6 by Thai herbal plants

Mechanism-based inhibition of the aqueous and ethanolic extract of Thai herbal plants on CYP3A4 and CYP2D6 activity were determined. The known mechanism-based inhibitors, troleandromycin (CYP3A4) and paroxetine (CYP2D6) was used as the positive control, while ketoconazole, erythromycin and clarithromycin, competitive inhibitors of CYP3A4 and quinidine and fluoxetine, competitive inhibitor of CYP2D6 were used as negative controls. The ethanolic extracts of P. amarus , M. hortensis , C. alata , K. parviflora, and Z. officinale showed significantly decreased in CYP3A4 activity after incubated with microsomes in the presence of NADPH in the time-dependent manner (data not shown). Similar results were observed with the aqueous extracts of P. amarus, K. parviflora, Z. officinale, S. leucantha, R. nasutus, G. pseudochina, C. asiatica and Cyanobacterium (data not shown). None of the ethanolic and aqueous extract of Thai herbs investigated in the present study showed significant time-dependent manner of CYP2D6 inhibition as observed with paroxetine (data not shown).

Discussions and Conclusions

In the present study, 18 Thai herbal plants were investigated for their in vitro inhibitory activity on human CYP3A4 and CYP2D6 using human hepatic testosterone 6β-hydroxylase and dextromethrophan O-demethylase as selective markers, respectively. Moreover, mechanism-based inhibition of these herbal extracts on these enzymes was characterized.

Results revealed that several of these extracts particularly the ethanolic extracts were potent inhibitors of CYP3A4 and CYP2D6. Some of these extracts inhibited CYP3A4 activity via mechanism-based inhibition. The evidence suggests that constituents in these herbs may be metabolized to reactive intermediates and inactivate CYP3A4. It has been shown in the previous studies that this type of inhibition can completely inactivate the metabolism of other drugs and cause serious adverse effects, which persist even after withdrawal of the inhibitors. Of course, the gene encoding the inactivated enzyme will produce the new enzyme, but this process may take several days, or at least, several hours to recover the enzyme activity to the sufficient level. During this time, lack of the drug-metabolizing enzyme increases the potential toxicity of the used drug. Therefore, health-care practitioners and patients should be awared when using these herbs with some prescribed drugs. The effects of these herbs *in vivo* need further investigation.

Acknowledgement

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P03 Antiglycation and Antiplatelet Aggregation Activities of Some Thai Medicinal Plants

Thanawat Kaewkamson[1], Patchareewan Pannangpetch[1], Bunkerd Kongyingyoes[1], Upa Kukongviriyapan[2]

Departments of [1]Pharmacology and [2]Physiology, Faculty of Medicine, Khon Kaen University
E-mail: patc_pan@kku.ac.th

Abstract

Introduction: Protein glycation- and free radical-induced tissue damages are ones of the proposed pathological mechanisms of hyperglycemia-induced diabetic complications such as cardiovascular disease and prothrombotic state. Platelets obtained from diabetic subjects were reported an increase in adhesiveness and an exaggerated aggregation. Disturbed carbohydrate and lipid metabolism as well as glycation process may lead to physicochemical changes in platelet membrane resulting in altered exposure of membrane receptor.

Objective: To obtain a medicinal herb for alleviating diabetic complications, we, therefore investigated the antiglycation and antiplatelet activities of some Thai medicinal plants which have already been scientifically revealed of their hypoglycemic activity: *Morus alba* Linn. (Mohn), *Combretum decandrum* (Sa-Kae-Krur), *Coscinium fenestratum* (Gaertn.) Colebr (Ham) and *Tinospora crispa* Miers ex Hook. F. & Thoms. (Bo-Ra-Ped), or antioxidant activity: *Syzygium gratum* (Wight) S.N. Mitra var. gratum (Pak-Mek), *Cratoxylum* subsp. *Pruniflorun* (Kurz) Gogel. T (Tew-Dang), *Cratoxylum formosum* Dyer.(Tew-Khow) and *Careya sphaerica* Roxb.(Kra-Don).

Materials and methods: Bovine serum albumin, glucose and plant extract were incubated together at 37°C for 2 weeks, then advanced glycation endproducts (AGEs) were measured by their fluorescence properties using fluorescence spectrophotometer. The platelet function was examined by using whole blood impedance aggregometer.

Results: We found that all hypoglycemic plants inhibited glycation effectively, while some of the antioxidant plants, Pak-Mek and Tew-Khow inhibited AGEs formation. Only Sa-Kae-Krur had a tendency to decrease platelet function.

Conclusion: It may be concluded that Mohn, Sa-Kae-Krur, Ham and Bo-Ra-Ped may possess potential for alleviating diabetic complications in addition to their antidiabetic activities.

Keywords: diabetic complications, antiplatelet, AGEs, antiglycation

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses, tissue or vascular damage ensues, leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases.

Hyperglycemia has an important role in the pathogenesis of diabetic complications by increasing protein glycation and the gradual build-up of advanced glycation endproducts (AGEs) in body tissues. These AGEs are formed on intra and

extracellular proteins, lipids, nucleic acids and possess complex structures that generate protein fluorescence and cross-linking. Protein glycation and AGEs are accompanied by increased free radical activity that contributes towards the biomolecular damage in diabetes (1).

Glycation that disturbs carbohydrate and lipid metabolism may lead to altered physico-chemical properties of platelet membranes which may further result in altered exposure of surface membrane receptors of platelets. Consequently, diabetic platelets are hypersensitive to agonists. These phenomena contribute to enhanced risk of small vessel occlusions and accelerated development of atherothrombotic disease of coronary, cerebral and other vessels in diabetes (2).

It is interesting that there are many medicinal plants in Thailand which possess antidiabetic or antioxidant activities. Antihyperglycemic plants are *Morus alba* Linn. (Mohn), *Combretum decandrum* (Sa-Kae-Krur), *Coscinium fenestratum* (Gaertn.) Colebr (Ham) and *Tinospora crispa* Miers ex Hook. F. & Thoms. (Bo-Ra-Ped). Antioxidant plants are *Syzygium gratum* (Wight) S.N. Mitra var. gratum (Pak-Mek), *Cratoxylum* subsp. *Pruniflorun* (Kurz) Gogel. T (Tew-Dang), *Cratoxylum formosum* Dyer.(Tew-Khow) and *Careya sphaerica* Roxb.(Kra-Don). Thus, if some of them have antiglycation or antiplatelet aggregation activities, they will be very useful to prevent diabetic complications when combining with antihyperglycemic drugs.

Methods

1) Plant extracts: The dried plants were minced and immersed with 50% ethanol for 3-5 days. The mixture was filtered through packed cotton and gauze. Ethanol was removed from the supernatant by using the rotary vacuum evaporator at 60°C under reduced pressure. The crude extract was freeze-dried by the lyophilizer.

2) Measurement of AGEs: Bovine serum albumin (10 mg/ml) in 50 mM phosphate buffer (pH 7.4) with 0.02 % sodium azide, to prevent bacterial growth, was added to a glucose (25 mM) and fructose (25 mM) solution. This reaction mixture was mixed with different concentrations of test samples. After incubating at 37°C for 2 weeks, the fluorescent reaction products were assayed on a fluorescence spectrophotometer with an excitation wavelength of 350 nm and an emission wavelength of 450 nm (3).

3) Measurement of platelet aggregation: Platelet aggregation was performed by using a whole blood impedance aggregometer. Five hundred microliters of donor whole blood with 3.2 % sodium citrate (1:10), was diluted with 0.9% NaCl in equal volume and incubated in socket of aggregometer at 37°C. After that, 20 µl of plant extract at various concentrations were added and incubated for 4 min further. Then, 10 µl of 1 mM ADP was added to induce platelet aggregation. Platelet aggregation was quantified as an increase in electrical impedance (Ω). Dipyridamole was used as a reference platelet inhibitor.

4) Statistical analysis: Results were presented as mean±S.E.M. The effect of plant extracts on AGEs formation and platelet aggregation were analysed by using Analysis of Variance (ANOVA). $P< 0.05$ was considered as statistical significance.

Results

As shown in Table 1, all antihyperglycemic plant extracts, Mohn, Sa-Kae-Krur, Ham and Bo-Ra-Ped, effectively inhibited AGEs formation with the potency of 1/100, 1/10, 1/5 and 1/10 times of aminoguanidine, respectively. For antioxidant plant extracts, Tew-Khow and Pak-Mek inhibited AGEs formation with equal potency of 1/50 of aminogunidine whereas Kra-Don showed only mild inhibitory effect. In

contrast, Tew-Dang, inhibited AGEs formation at low concentrations (1-3 μg/ml) but increased at high concentrations (10-100 μg/ml).

The four plant extracts, Mohn, Sa-Kae-krur, Pak-Mek and Tew-Khow with strong antiglycation effect, were selected to investigate antiplatelet activity. While Sa-Kae-Krur had a tendency to inhibit ADP-induced platelet aggregation, the other three plants tended to enhance it (Fig.1a-e). However, those events were not statistically significant. Dipyridamole, a standard antiplatelet drug, showed 50% inhibition at 200 μg/ml.

Table 1 Effect of some Thai medicinal plant extracts on AGEs formation

Plant Extracts	Antiglycation Activity	
	IC_{50} (μg/ml)	Maximum Inhibitory Effect
Mohn	27.5 ±5.9	98.6 ± 1.2
Sa-Kae-Krur	1.9 ± 0.3	89.7 ± 7.3
Ham	0.9 ± 0.1	95.5 ± 1.6
Bo-Ra-Ped	2.0 ± 0.0	66.4 ± 5.0
Pak-Mek	7.3 ± 1.4	100.0 ± 0.0
Tew-Khow	8.1 ± 3.1	84.9 ± 13.1
Aminoguanidine	0.185 ± .08	100.0 ± 0.0

AGEs: Advanced glycation endproducts; IC: Inhibitory concentration; Number of experiments=4

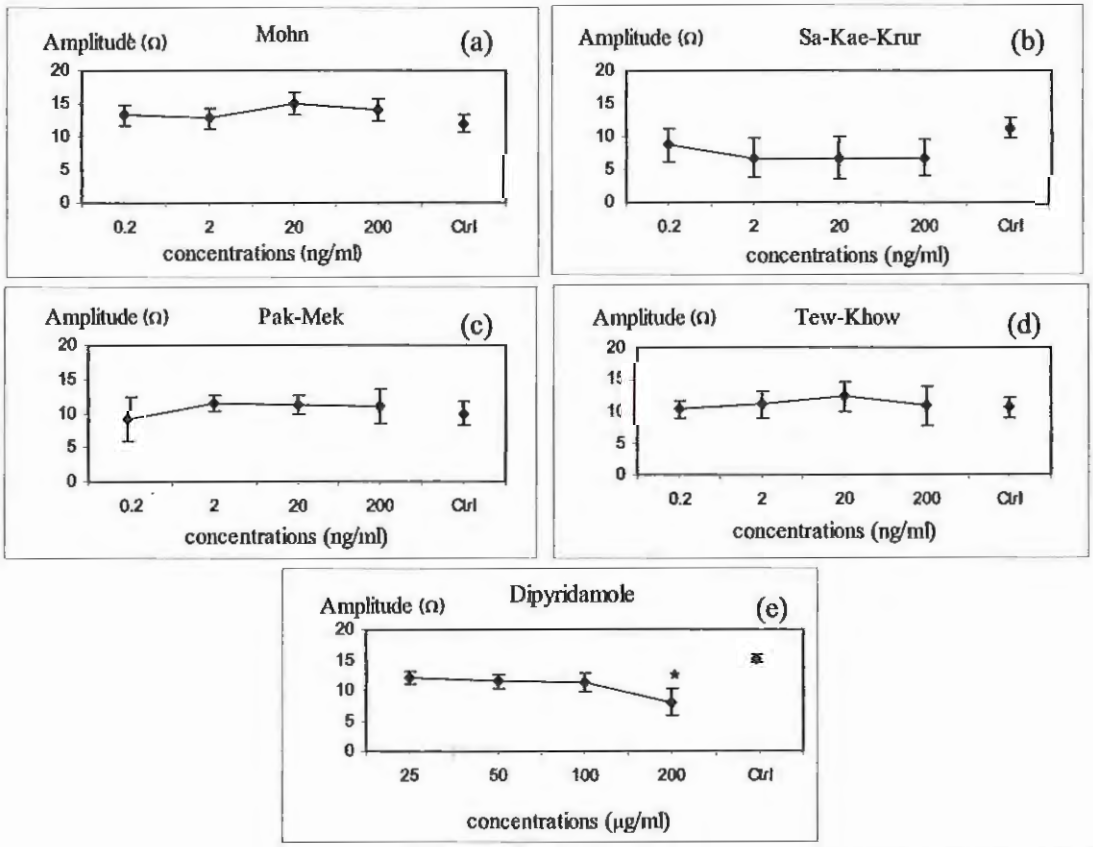

Figure 1a-e Effect of Mohn (a), Sa-Kae-Krur (b), Pak-Mek (c), Tew-Khow (d) and dipyridamole (e) on ADP-induced human platelet aggregations. Sa-Kae-Krur showed a tendency to inhibit whereas Mohn, Pak-Mek and Tew-Khow tended to enhance platelet aggregations. However, theses were not statistically significant.
Number of experiments=4, *: $P<0.05$ as compared to control, ctrl: control

Discussion

AGEs are formed when a carbonyl moiety of a reducing sugar condenses with a reactive amino group in a target protein. They accumulate slowly in the body as a function of age, but more rapidly in individuals with diabetes mellitus. Recent studies have indicated that some radical species, including hydrogen peroxide, superoxide anion radicals, and singlet oxygen, participate in AGEs formation (4), and that antioxidant and radical scavengers inhibit these processes (5). In addition, AGEs induce platelets to be hypersensitive to agonists. Accumulating evidence indicates that platelets from diabetic patients are hyperactive. High glucose level enhanced ADP- and thrombin receptor-activating peptide (TRAP)-induced platelet P-selectin expression, and TRAP-induced platelet fibrinogen binding (6). Endothelial abnormalities also play a significant role in the enhanced activation of platelets and clotting factors seen in diabetic patients (6).

It is interesting that antidiabetic plants, Mohn, Sa-Kae-Krur, Ham and Bor-Ra-Ped can decrease the in vitro glycation process indicating that these antidiabetic plants may provide activities to alleviate diabetic complications in addition to their antihyperglycemic activities. Pak-Mek and Tew-Khow can also diminish AGE formation which possibly at least via their antioxidant activities.

None of the tested plant extracts affect healthy human platelet aggregation induced by ADP. However, this may not be relevant to diabetic patient platelets which are in hyperreactive situation. To solve this problem, we have a plan to investigate the effect of antidiabetic plants on diabetic rat platelets. Nevertheless, we may say that Mohn, Sa-Kae-Krur, Pak-Mek and Tew-Khow do not distrurb platelet function of healthy person.

Conclusion

Through their antiglycation activities, Mohn, Sa-Kae-Krur, Ham and Bo-Ra-Ped may have diabetic complication alleviation effects in addition to their antidiabetic activities.

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P04 The Relationship between Pharmacokinetics of Deferiprone (L1) and Iron Kinetics in β-Thalassemia/Hemoglobin E Patients

Totsapol Jirasomprasert [1], Lie Michael George Limenta[1], Supeenun Unchern[1], Udom Chantharaksri[1], Suthat Fucharoen[2] and Noppawan Phumala Morales[1]

[1]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand.*
[2]*Thalassemia Research Center, Institute of Science and Technology for Research and Development, Mahidol University, Nakornphathom, Thailand*
E-mail: scnpm@mahidol.ac.th

Abstract

Introduction: Deferiprone (L1) is an orally active iron chelator used in patients with iron overloading. β-thalassemia/HbE (β-thal/Hb E) patients show high variability in the degree of iron accumulation in various organs, depending on the disease severity. Iron overload may alter the pharmacokinetic profile of deferiprone.

Objective: The aim of this research is to study the effects of iron overloading status on the pharmacokinetics of deferiprone.

Materials and methods: The relationships of serum profile and urinary excretion of deferiprone and non-transferrin bound iron (NTBI) were determined in eleven normal subjects and twenty-one β-thal/Hb E patients. Following an overnight fasting, a single oral dose of deferiprone (25 mg/kg) was administrated. Blood samples were obtained at pre-dose and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min after dosing. Urine samples were collected at 2, 4, 8, 12 and 24 hours after dosing. Non-glucuronide deferiprone in serum and urine samples were determined by high performance liquid chromatography (HPLC). Serum non transferrin bound iron (NTBI) and urinary iron excretion (UIE) were determined by a colorimetric method.

Results: The results demonstrated that pharmacokinetic parameters of deferiprone in the patients were significant different from those of normal subjects. Thalassemic patients had lower maximum concentration (Cmax) and area under the concentration time curve ($AUC_{0\text{-}\infty}$) with longer half-life ($elt_{1/2}$) and higher volume of distribution (Vd). The levels of serum NTBI and deferipone were elevated. The ratio of serum deferiprone:NTBI and amount of iron excretion in urine were dependent on basal NTBI patients.

Conclusion: In heavily iron overloaded patients, the ratio of serum deferiprone:NTBI was lower than 3 after 300 minutes of deferiprone administration, suggesting the tendency of iron toxicity in these patients. These results may be useful for a proper design of a dosage regimen of deferiprone in patients with different degrees of iron overload status.

Key words: deferiprone/ iron overload/ pharmaokinetic/ β-thalssemia/hemoglobin

Introduction

Thalassemia is one of the common iron overload diseases caused by mutations in the globin genes. The lack of effective hemoglobin synthesis leads to anemia that is corrected by blood transfusions and this along with increased gastrointestinal iron uptake leads to iron overload. Because of the saturation of transferrin, there is an increasing amount of low molecular weight complexes of iron in blood which available in the catalyzing of free radical formation and damage to tissue. In order to eliminate iron toxicity and reduce excess iron in the body, patient should properly receive iron chelator.

Deferiprone is an orally active iron chelating drug which has been available for clinical use in 1995. It is a bidentale; need 3 molecules to bind one iron atom at pH 7.4. It has been reported that deferiprone 75 to 100 mg/kg/day can induce negative iron balance and decrease tissue iron in the patients but there are many factors that could modified the effect of deferiprone including the degree of iron overloading, duration, dosage and degree of compliance with therapy so the drug could be ineffective in some patients. The adverse effects of deferiprone such as arthritis, agranulocytosis, arthropathy, gastrointestinal symptoms, increased ALT levels and progression of hepatic fibrosis can be found. However, the optimum dosage, long term efficacy and mechanism of toxicity remain unclear.

This research was aimed to determine the effect of iron overload in β-thal/HbE patients on the pharmacokinetic parameters of deferiprone. The relationship between serum profile of deferiprone and NTBI as well as urinary deferiprone and iron excretion are also studied. To evaluate the toxicity potency of deferiprone in blood circulation, the ratio of deferiprone to NTBI will be calculated. The results may be useful in optimizing dosage regimen of deferiprone in the patient with different degree of iron overloading.

Methods

Subjects Eleven Thai normal subjects and twenty-one β-thal/HbE patients were enrolled in the study. According to hemoglobin level, age at disease presentation, age at first transfusion, frequency of transfusion, degree of hepatosplenomegaly, and growth retardation, 11 patients (six males and five females, 31.8 ± 7.5 years old) and 10 patients (five males and five females, 27.7 ± 8.6 years old) were categorized as mild to intermediate and severe groups, respectively. None of these patients had blood transfusions in one month prior to blood sampling and or had taken any medications except their daily folic acid supplementation. The study protocol was approved by the Ethics Committee of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Pharmacokinetic study After an overnight fast for at least 8 hours, the subjects were inserted with catheter into a vein at the dorsal surface of the hand for blood samplings and flush normal saline containing of heparin to prevent blood clotting. A single dose of deferiprone 25 mg/kg was administered orally followed by 200 ml of drinking water. The subjects had breakfast after intake the drug for 2 hours. Venous blood samples (5 ml) were collected at pre-dosing and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min after dosing. Serum was immediately separated and stored at -20°C until the time of analysis. Urine collections in 24-hours were collected at intervals (2, 4, 8, 12 and 24 hours) after dosing. Urine samples were measured volume and stored into a tube with tight cap then frozen at -20°C until analysis.

Quantitative analysis of NTBI and UIE Serum NTBI and urinary iron excretion (UIE) were determined by colorimetric method using thioglycolic acid (TGA) and bathophenanthrolinedisulfonic acid (BPT) as a reducing agent and chromogen, respectively.

Quantitative analysis of non-glucuronide conjugated form of deferiprone Serum and urine samples were filtered using ultrafiltration technique then directly injected to HPLC system which consisting of high pressure pump, autosampler injector and dual wavelength absorbance detector. Mobile phase was composed of methanol and sodium dihydrogen phosphate (7:93 (v/v) for serum sample and 10:90 (v/v) for urine sample (10 mM, containing 2 mM EDTA, pH 3.0 adjusted with phosphoric acid). The detector was set at 280 nm.

Results

Serum profiles and pharmacokinetic parameters of single dose deferiprone (25 mg/kg BW) in normal subjects and thalassemic patients were demonstrated in Figure 1 a. and Table 1, respectively. Serum NTBI level was simultaneously increased with deferiprone. In normal subjects, the NTBI was slightly increased and reached its peak concentration within 45 min. Concentration of NTBI in the both groups of patients were significantly higher than that of normal individuals. The peak concentration levels were found between 120 to 180 min (Figure 1 b).

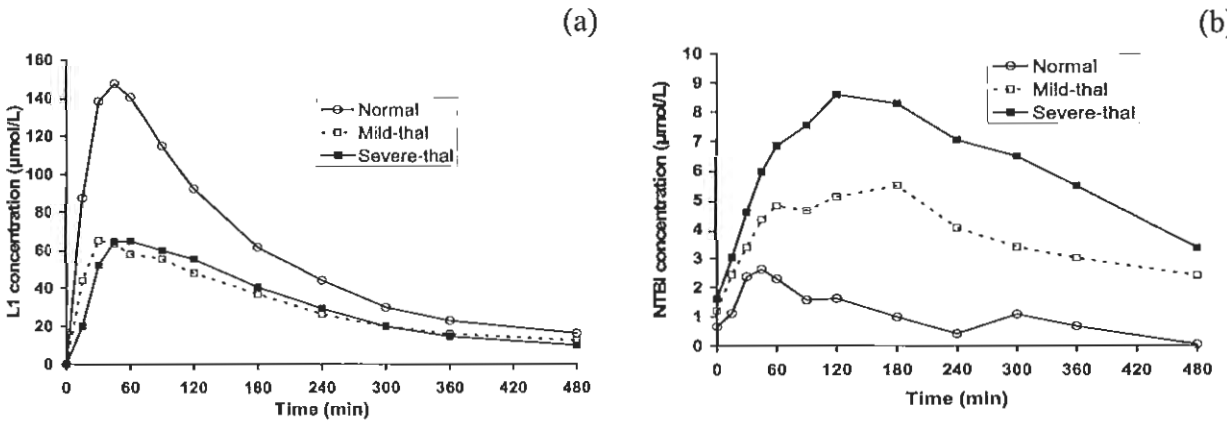

Figure 1: Mean concentration of non-glucuronide deferiprone (a) and NTBI (b) in serum sample of normal (○), mild to intermediate thalassemia (□) and severe thalassemia (■) after administration of deferiprone 25 mg/kg body weight

Table 1: Pharmacokinetic parameters of deferiprone in serum after single oral administration of 25 mg/kg body weight in normal subjects and thalassemic patients

	Normal n=11	β-thalassemia/Hemoglobin E	
		Mild to intermediate n=11	Severe n=10
Tmax (min)	42.3 ± 21.0	57.3 ± 34.1	57.0 ± 29.0
Cmax (µg/ml)	24.8 ± 7.5	12.0 ± 4.4 [a]	11.7 ± 3.6 [a]
elt $_{1/2}$ (min)	137.0 ± 18.1	325.0 ± 162.0 [b,1]	195.4 ± 84.1 [1]
$AUC_{0->\infty}$ (min.µg/ml)	4169.9 ± 913.2	2926.4 ± 848.6 [b]	2487.7 ± 574.0 [b]
Vdapp (ml/kg)	1266.2 ± 224.6	3952.2. ± 1364.1 [a]	2920.3 ± 1417.1 [b]
Clapp (ml/min)	375.9 ± 100.5	466.4 ± 166.5	449.0 ± 116.8

Data are presented as mean ± SD
[a] $p<0.0001$, [b] $p<0.01$ compared with normal subjects
[1] $p<0.05$ compared with mild to intermediate

Urinary iron excretion is shown in Figure 2 a. In thalassemia, all of deferiprone, together with iron was rapidly excreted in urine within 12 hr. The 24 hr urinary iron excretion was 58.8 ± 68.4 and 84.3 ± 43.1 µmol in mild to intermediate and severe patients, respectively. Good correlation was found between non-transferrin bound iron at time zero ($NTBI_0$) and 24 hr urinary iron excretion (UIE) (Figure 2 b). Although non-glucuronide deferiprone was also found, iron was not detected in urine of normal subjects.

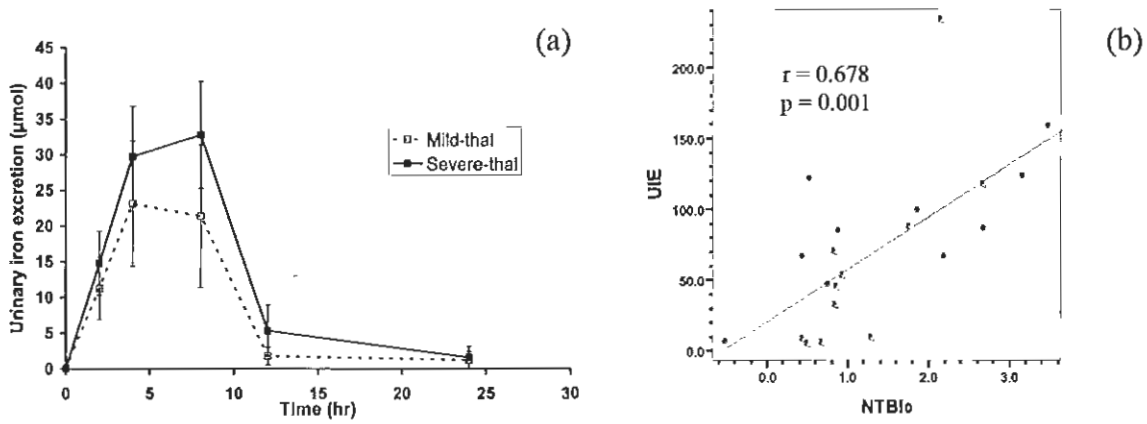

Figure 2: Amount of iron excretion in urine of the subjects after deferiprone administration (a) and correlation between $NTBI_0$ and UIE (b)

The concentration ratios of deferiprone to non-transferrin bound iron (L1: NTBI) and deferiprone to urinary iron excretion (L1: UIE) in serum and urine samples were determined at each time point. At all time points, the ratios were higher than 3 in most of the subjects. However, at 300-480 min after deferiprone administration, there were 3 of 11 patients in mild to intermediate group and 5 of 10 severe patients had L1: NTBI ratios lower than 3.

Discussion

The pharmacokinetic parameters of deferiprone in the present study showed that this drug was rapidly absorbed after an oral administration. Time to reach maximum concentration (Tmax) was 15 to 90 minutes. Maximum concentration, area under the serum concentration time curve and volume of distribution were significantly different between normal and thalassemia group. The elimination half-life of deferiprone was in the range of 2-4 hr, suggesting that the daily amount may have to be given every 4 hr to maintain an effective level of deferiprone.

In thalasemia, iron toxicity can be found when the loads of iron in the tissue exceed the binding capacity of ferritin in the cell and of transferrin in the plasma. This results in accumulation of free or non-transferrin bound iron (NTBI) which can damage the tissue so it must be chelated out of the body. This study showed that deferiprone can induce NTBI excretion and the maximum urinary iron excretion was found within 4-8 hr after administration and the level of iron excretion in severe group was higher than mild to intermediate group. The ranges of iron excretion have a wide variation which may relate partly to a wide range in body iron stores, with iron excretion being greater in those with greater body iron burden. However, the drug does not appear to chelate iron in normal subjects.

The ratios of deferiprone to non-transferrin bound iron (L1: NTBI) in the serum of normal subjects at all time points were higher than 3, while in mild to intermediate and severe thalassemia, the ratios were greater than 3 until 300 min. After that, some patients had the lower ratios. In *vitro* studies investigating the effects of deferiprone concentrations on iron-mediated ascorbate oxidation and deoxyribose degradation indicate that the L1: iron ratio must be at least 3:1 to inhibit free radical generation. At lower deferiprone concentrations, free radical generation is increased. The results of this study implied that after 300 min, deferiprone was inadequate to maintain the optimum L1: iron ratio and probably resulted in iron toxicity.

Acknowledgement

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P05 Pharmacology Learning Achievement in Medical Cadets: the Comparison Between Conventional and Integration Courses

Nisamanee Satyapan, Borpit Klangkalya, Supen Patarakitvanit, Supatta Temboonkiat, Jeeranut Tankanilert, Sarawut Jindarat , Janeyuth Chaisakul

Department of Pharmacology, Phramongkutklao College of Medicine, Bangkok
E-mail: nsatyapan@yahoo.com

Abstract

Introduction: It is not known whether the change in the discipline oriented and lecture-based (conventional) to an integration and problem-based courses will affect the learning achievement in pharmacology topics or not.

Objective: The essential knowledge in pharmacology between two medical cadet classes, one had learnt in conventional and the other in an integrated courses over the transition period (year 2003-2004) were compared.

Materials and methods: One hundred multiple choice examination questions covering all the topics in pharmacology were selected. The degree of difficulties (p) of each question for both groups of students were analyzed and compared.

Results: The results showed that the average degree of difficulties for all sets of the questions were not significantly different between the two student groups, indicating no difference in pharmacology knowledge. The students attending integrated course had better performance in some topics: i.e., antimicrobial agents. This may be due to a better understanding in the correlation study with microbiology topics and with more emphases on clinical applications in the integration course.

Conclusion: Several factors may be involved in the student learning achievement and more extensive study should be encouraged.

Key Words: pharmacology learning, conventional course, integration course, medical cadets

Introduction

Faculties at medical schools that employ problem-based learning (PBL) have been shown to have positive perceptions, but not all schools are in a position to adopt PBL on a larger scale [(1)]. Phramongkutklao College of Medicine has changed the curriculum from discipline oriented and lecture-based (conventional) to integration and problem-based courses since 2003. It is not known whether these changes will affect the learning achievement in pharmacology topics or not. This study was designed to compare the essential knowledge in pharmacology between two medical cadet classes who had learnt in conventional and integration courses during the transition period (year 2003-2004).

Materials and Methods

Retrospective study was performed using one hundred multiple choice examination questions, employed during the year 2003-2004. The questions were selected to cover all the topics in pharmacology teaching. Each of these questions which were used to evaluate the knowledge in both groups of students was either the same or paralleled question. The degree of difficulties (p) of each question for both groups of students were analyzed and compared, statistically.

Results and Discussion

All (100) questions were classified into 3 sets. They are recall (51), interpretation (25) and application (24).

The data were analyzed by using Independent Sample T Test at 95 % confidence interval. It was found that the average p for all sets of the questions were not significantly different between the two student groups (P-value > 0.05), indicating no difference in pharmacology knowledge (Table 1).

Besides, the students attending integration course have better performance in some topics (P-value < 0.05): i.e., antimicrobial agents (Table 2). This significant finding may be due to a better understanding in the correlation study with microbiology topics and with more emphases on clinical applications in the integration course.

Conclusion

Hybrid curriculum in this study tends to have a positive effect on students' abilities and of the curriculum as does PBL. However, several factors may be involved in the student learning achievement in pharmacology, as well [2,3,4,5]. More extensive study should consequently be encouraged.

Acknowlegement

We thank Supak Saengow for her help with the statistics.

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Table 1: Average Degree of Difficulty in Conventional and Integration Course Classified as Recall, Interpretation and Application

	Conventional (p)	**Integration (p)**	**P-value**
	Mean ± SD	Mean ± SD	
Recall	0.67 ± 0.21	0.73 ± 0.19	0.120
Interpretation	0.68 ± 0.16	0.69 ± 0.23	0.780
Application	0.66 ± 0.23	0.74 ± 0.21	0.222
Total	0.67 ± 0.21	0.72 ± 0.20	0.059

Table 2: Average Degree of Difficulty in Conventional and Integration Course, Classified by Topic in Pharmacology Teaching

Group	Titles	**Conventional (p)**	**Integration (p)**	**P-value**
		Mean ± SD	Mean ± SD	
1.	Introduction to pharmacology (Disposition, etc)	0.71 ± 0.18	0.60 ± 0.19	0.124
2.	Antimicrobial agents	0.55 ± 0.18	0.68 ± 0.18	0.018*
3.	Neuropharmacology (ANS ,CNS drugs, etc)	0.68 ± 0.23	0.74 ± 0.22	0.330
4.	Cardiovascular drugs	0.73 ± 0.18	0.80 ± 0.15	0.227
5.	Respiratory drugs	0.77 ± 0.23	0.89 ± 0.08	0.255
6.	GI drugs	0.58 ± 0.22	0.76 ± 0.15	0.114
7.	Endocrine drugs	0.68 ± 0.22	0.69 ± 0.33	0.944
8.	NSAIDs	0.86 ± 0.047	0.77 ± 0.16	0.324

* P-value < 0.05

P06 Effects of Synthetic CCK Antagonist on Animal Behavior

Janeyuth Chaisakul[1], Jintana Sattayasai[2], Borpit Klangkalya[1], Eric Lattman[3] Yodchai Boonprakob[2], Thishnapha Vudhironarit[1]

[1]*Department of Pharmacology, Phramongkutklao College of Medicine, Bangkok*

[2]*Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen*

[3]*The School of Pharmacy, Aston University, Aston Triangle, Bermingham B4 7ET, UK*

E-mail: najane_kaouthia@hotmail.com

Abstract

Introduction: Localization of CCK and GABA in the cortex, hippocampus and the CCK projection from prefrontal cortex to striatum suggest the possible roles of CCK in many psychiatric disorders, including anxiety, depression and attention deficit and in the negative symptoms and cognitive deficits of schizophrenia.

Objective: The present study was carried out to screen the central nervous system activities of BzoIb which is one of the synthetic cholecystokinin (CCK) antagonists.

Materials and methods: In the animal model, BzoIb (either 1 or 5 mg/kg) was intraperitoneally injected into mice and the effects of this agent as an antidepressant (forced swimming model), antianxiety (elevated pus maze) and motor power were tested in comparison with desipramine and diazepam.

Results: The results showed that BzoIb induced a significant increase ($P<0.05$) in the time that the mice spent in an open arm after administration, as well as a significant decrease in immobility time. However, a significant change in number of arm entries was not observed.

Conclusion: This study showed that the synthetic CCK antagonist-BzoIb could antagonize the depression and anxiety in mice.

Key words: Cholecystokinin (CCK), Antidepressant effect, Anxiolytic effect

Introduction

Cholecystokinin (CCK) belongs to the family of gut-brain peptide. The peptide CCK was initially discovered in the gastrointestinal tract and had been shown to mediate gallbladder contraction and secretion of pancreatic enzymes. Surprisingly, CCK was described in the mammalian central nervous system (CNS) (1) and believed to be one of the most widespread and abundant neuropeptide in the CNS. Localization of CCK and GABA in the cortex, hippocampus and the CCK projection from prefrontal cortex to striatum suggest the possible roles of CCK in many psychiatric disorders, including anxiety, depression and attention deficit and in the negative symptoms and cognitive deficits of schizophrenia.

To date, two CCK receptors have been identified, namely, the CCK_A and CCK_B receptors subtypes (2). Several studies indicated that stimulation of either one of the two CCK receptor subtypes expresses a variety of physiological and behavioral effects. CCK_A receptor has been found in the GI tract and certain areas in the brain, e.g. area postrema and vagus nerve complex. CCK_B receptors are much more widely distributed in the CNS especially in cortical and limbic areas. Many studies have reported that CCK induced panic like attacks in healthy volunteers (3) and patients with panic disorder (4) shortly after injection. These results have guided to the conclusion that, possibly, CCK_B receptors in the brain are involved in the regulation

of anxiety. In the recent years, specific and highly potent CCK antagonists have been developed. The availability of these compounds has prompted examinations into the functional roles of CCK in the brain. This has guided new possibilities for the treatment of CNS disorders (5-7). In this study, certain synthetic CCK antagonists were investigated for CNS effects, especially those involved with anxiety, depression and effects on motor activities in mice.

Methods

All experiments were performed at room temperature. Synthetic CCK antagonist (Bzolb) was obtained from School of Pharmacy, Aston University, UK and dissolved in 5% DMSO. Mice weighing 25-45 g were injected intraperitoneally with either 5% DMSO as the control group or test substances (1 or 5 mg/kg) and were divided into 5 groups (n = 10 per group). For determining the effects of synthetic compound, desipramine (10 mg/kg) and diazepam (1mg/kg) were respectively used as a positive control for antidepressant-like and anxiolytic-like effects. Animals were assessed for their behavioral changes at 30 minutes after the treatment. The forced swim test and elevated plus maze were used for screening of antidepressant-like and anxiolytic–like effects, respectively. Muscle power assessment was done by using the wire mesh-grasping test. All data were expressed as mean $\pm$ SD value. The differences among various groups were compared by ANOVA. A level of p-value < 0.05 was considered significant.

Results

For the anxiolytic-like effect, the synthetic CCK antagonist (Bzolb), at either 1 or 5 mg/kg could induce a significant ($P < 0.05$) increase in time spent in the open arm after administration (Figure 1). However, there was no change in the number of arm entries. Bzolb also possessed antidepressant-like effect as a significant decrease in the immobility time could be observed in animals treated with Bzolb at 1 or 5 mg/kg in a comparable manner with 10 mg/kg desipramine (Figure 2). In the wire mesh-grasping test, no effect on muscle power could be seen at 30 min after the injection of Bzolb, while diazepam could significantly reduce the time animal hang on the sieve (Table 1).

Discussion

CCK is known to play important roles in expressing the symptopathology of the stress responses such as depression and anxiety-related disorder. In this study, Bzolb at the doses of 1 and 5 mg/kg showed both antidepressant-like and anxiolytic-like effects, evidence which gave support to the roles of CCK system in the animal models. Among the experimental animal models used for testing the CNS effect of compound, the forced swim test and the elevated plus maze are commonly used models as they are easy to perform and no need for any expensive instruments.

Conclusion

The results from this study showed the antidepressant-like and anxiolytic-like effects of Bzolb which is a synthetic CCK antagonist. Interestingly, it may provide advantage as anxiolytic and anti-depression agent.

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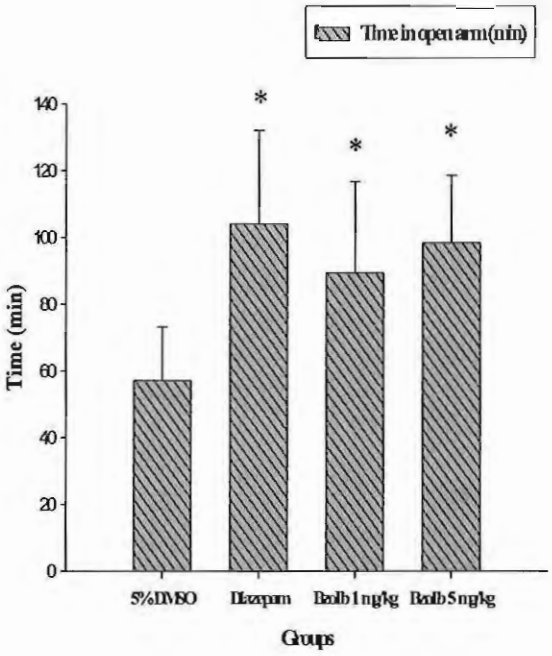

Figure 1 Time spent in open arm of mice at 30 min after intraperitoneal injected with 5% DMSO, diazepam (1 mg/kg) or BzoIb (1 or 5 mg/kg). *Significant difference ($P<0.05$) when compared to the group of 5%DMSO.

Figure 2 The immobility time of mice at 30 min after treated with either 5% DMSO, desipramine (10 mg/kg) or BzoIb (1 or 5 mg/kg). * Significant difference (P<0.05) when compared to the group of 5% DMSO.

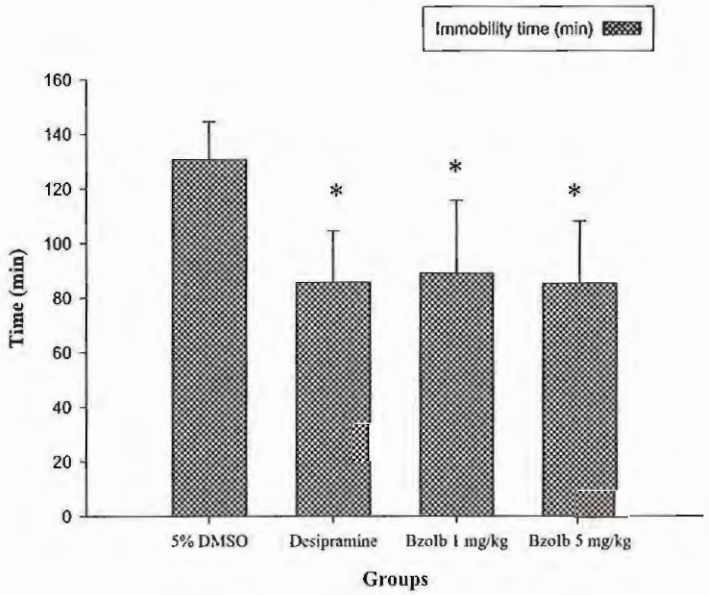

Table 1: The muscle power in the wire mesh-grasping test expressed as the percentage of time hang on the sieve and the number of arm entries of mice in the elevated plus maze when compared to the control.

Groups	5% DMSO	Diazepam	BzoIb 1 mg/kg	BzoIb 5 mg/kg
The number of arm entries (time)	21.22±2.77	27.00±4.06*	21.56±4.28	18.44±2.13
% of muscle power	99.17±2.63	74.50±19.53*	94.33±11.94	99.33±2.11

* Significant difference (P<0.05) when compared to the group of 5%D MSO.

P07 Hypolipidemic and Antiatherosclerotic Effects of *Curcuma comosa* on High Cholesterol-fed Rabbits

Yupin Sanvarinda[1], Laddawal Phivthong-ngam[2], Piyanee Ratanchamnong[1], Pawinee Piyachaturawat[3]

[1] *Department of Pharmacology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400.*
[2] *Department of Pharmacology, Faculty of Medicine Srinakarinwirot University, Sukumvit Road 23,. Bangkok 10110*
[3] *Department of Physiology, Faculty of Science, Mahidol University, Rama 6 Road Bangkok 10400.*
E-mail: scysv@mahidol.ac.th

Abstract

Introduction: Hypercholesterolemia plays pivotal role in the pathogenesis of atherosclerosis. Recent study reported that anti-inflammatory agents reduced plaque formation and improved vascular function in hypercholerolemic animal. *Curcuma comosa* (*C. comosa*) is used in folk medicine as anti-inflammatory agent.

Objective: We investigated the effect of *C. comosa* on cholesterol (C) levels and plaque formation in cholesterol-fed rabbits.

Materials and methods: Rabbits were fed with C, C + simvastatin C + crude powder of *C. comosa* or normal rabbit chow for 12 weeks. Plasma total C and triglyceride concentrations were analyzed every 4 weeks. Twelve weeks after the treatment the rabbits were sacrificed, arterial plaque formation was determined.

Results: We found that crude powder of *C. comosa* significantly lowered the cholesterol levels and reduced plaque formation.

Conclusion: The results indicate that *C. comosa* possesses hypocholesterolemic effect and retards atherosclerotic plaque formation. Our results may provide an alternative for the treatment of hyperlipidemia.

Key words: *Curcuma comosa*, hypercholesterolemic rabbits, plaque formation

Introduction

Cardiovascular disease is the leading cause of death among population in developed nations including Thailand. It is well recognized that the incidence of cardiovascular disease in men is higher than in women, however, this incidence increases substantially in women after menopause, purportedly due to the loss of estrogen's protection. The role of estrogen deficiency in cardiovascular disease (CVD) was further indicated by preclinical studies demonstrated that estrogen could inhibit atherosclerosis (1). In addition, it showed that women taking estrogen replacement therapy experienced a significant decline in levels of low-density lipoprotein (LDL) while the high-density lipoprotein (HDL) increased (2). Nevertheless, a recent study reported the risks of estrogen therapy, which include hypertriglyceridemia, endometrial hyperplasia, tumorigenesis, hypercoagulable states, and angiogenesis which is the fundamental process necessary for tumorigenesis as well as atherosclerotic progression.(3) Given the demonstrated risks of estrogen, the search for alternatives has been done. Favorable effects of phytoestrogens on lipid profiles, vascular reactivity, thrombosis and cellular proliferation have been reported (4). Common and significant source of phytoestrogens are soybeans, cereal oilseeds and some medicinal plants such as *C. comosa* and *Pueraria mirifica* .

C. comosa is an indigenous plant of Thailand. Previous study demonstrated that agent possesses anti-inflammatory action prevents atherosclerotic plaque and improves vascular function in hypercholesterolemic animals (5). In addition, recent study reported the inhibitory effect of *C. comosa* on NO and cytokines production in LPS activated microglia (6). Other previous studies demonstrated the estrogenic activity (7) and hypocholesterolemic effects of *Curcuma comosa* in hamster (8). Based on these results, we therefore, investigated the hypocholesterolemic and the antiatherosclerotic effects of CC in hypercholesterolemic rabbits.

Methods:

Thirty two male New Zealand White rabbits initially weighing 1.5-2.0 kg. were used in this study. The animal study conformed to the guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care (1993; Vol 1; http://www.ccac.ca/). After a 4-week period of acclimatization, the rabbits were exposed to dietary treatment for a period of 12 weeks. The rabbits were randomly divided into 4 groups:

Group 1 Normal rabbit chow throughout the experimental period (control group)

Group 2 A a diet containing 0.5% cholesterol throughout the experimental period (cholesterol group)

Group 3 A diet containing 0.5% cholesterol + 5 mg simvastatin throughout the experimental period (simvastatin group)

Group 4 A diet containing 0.5% cholesterol + 4 00 mg/kg crude powder of *C. comosa* throughout the experimental period (*C. comosa* group).

Food and water were supplied *ad libitum* for all groups. Prior to the beginning of the experiment and at every 4 weeks thereafter, blood samples were drawn by puncture of the central ear vein for analysis of lipid profiles. At the end of the feeding period, blood samples were taken and animals were sacrificed. The internal carotid artery was isolated and used for histological examination of plaque formation.

Results

The plasma concentrations of total cholesterol in all groups of rabbits are shown in Table 1. Total plasma cholesterol at baseline in all groups of rabbits is not significant difference. The total cholesterol concentration in the control group showed no significant difference during the whole experimental period. In contrast, plasma total cholesterol levels in 0.5% cholesterol feeding group increased to 1185.5 ± 156.3 mg/dL at the end of experimental period. The total cholesterol levels in the simvastatin and *C. comosa.* treated groups significantly lower than the cholesterol group. The difference was evident after 4 weeks of treatment. The changes in LDL-cholesterol and HDL-cholesterol went parallel to the plasma total cholesterols in all groups of animals. When compared to cholesterol group, at the same time point, simvastatin and *C. comosa* significantly decreased the levels of both LDL- and HDL-cholesterol

After 12 weeks of dietary intervention, no intimal thickening of internal carotid artery cross-sections was evident in the control group. However, the intimal area of the cholesterol group was significantly increased, whereas the intimal area in the simvastatin and *C. comosa* groups significantly decreased and was significantly different from that of the cholesterol group. (Table 2)

Discussion

The results of this study clearly demonstrate that crude powder of *C. comosa.* possesses hypocholesterolemic effects and can reduce arterial plaque formation in rabbits fed with high cholesterol diet. These effects may result from the action of

phytoestrogens present in this plant. Moreover, the other compounds that have anti-inflammatory or antioxidative actions may also involve in these effects.

Conclusion

The results of this study indicate that *C. comosa* has a potential to be developed as a therapeutic compound for prevention of cardiovascular disease.

Acknowledgements

This study was partially supported by a grant from the National Research Council of Thailand

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

Group of rabbits	Time Period (weeks)			
	0	4	8	12
Total cholesterol (mg/dL)				
Control	45.0 ± 5.1	49.0 ± 4.2	41.2 ± 4.2	37.1 ± 5.1
Cholesterol	40.6 ± 3.1	714.0 ±89.7 *	1012.0±115.2*	1185.5±156.3*
Chol+Simvas	43.7 ± 1.2	470 ±124.05*†	390.7 ± 102.2 *†	372.3 ± 106.5*†
Chol+Crude CC.	43.6 ± 7.09	345.7 ± 88.2*†	378.9 ± 107.4*†	383.4 ± 118*†
LDL cholesterol (mg/dL)				
Control	23.7 ± 2.2	20.1 ± 1.67	19.8 ± 3.5	18.0 ± 2.52
Cholesterol	19.8 ± 2.1	657.5 ± 112.5*	941.5±46.5*	1005.0±100.2*
Chol+Simvas	20.9 ± 3.5	379.1±72.7*†	342.4±69.4*†	292.0±54.5*†
Chol+Crude CC	18.5 ± 5.21	286.2±75.9*†	334.0±96.8*†	342.9±109.0*†
HDL cholesterol (mg/dL)				
Control	34.3 ± 3.1	33.7±5.7	30.2 ± 5.8	26.9±3.2
Cholesterol	30.9 ± 7.5	181 ± 60.8*	246 ± 28.9*	302 ± 25.5*
Chol+Simvas	37.0 ± 4.4	123.9 ± 44.7*†	125.2 ± 64.4*†	150.7 ± 51.9*†
Chol+Crude CC	36.4 ± 4.5	88.4 ± 17.7*†	114.5±32.9*†	105.8±32.5*†
Triglycerides (mg/dL)				
Control	71.0 ± 7.1	61.9 ± 7.5	76.2 ± 8.2	75.5 ± 13.5
Cholesterol	80.2 ± 10.2	147.0 ± 38.2*	147.6±32.8*	197.3±45.0*
Chol+Simvas	77.8 ± 9.8	79.2 ± 28.6†	72.2 ± 29.3†	78.0 ± 23.4†
Chol+Crude CC	79.7 ± 13.9	72.1 ± 12.5†	73.6 ± 16.5†	73.1 ± 10.0†

No. of rabbits per group = 8 All values are mean ± SEM
* P< 0.05 compared to control group
† P< 0.05 compared to cholesterol group
CC = *C. comosa*

Table 2. Plaque formation on the left internal carotid artery in various groups of rabbits

Group of rabbits	% Plaque formation
Control	0
Cholesterol	26.8 ± 7.94*
Cholesterol + Simvastatin	1.7 ± 1.98 *†
Cholesterol + Crude C. comosa	8.9 ± 2.71*†

* P< 0.05 compared to control group
† P< 0.05 compared to cholesterol group

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P08 Free Radical Scavenging Activity of Curcumin and Its Derivatives

Srisuporn Sirijaroonwong [1], Supeenun Unchern[1], Noppawan Phumala Morales[1] and Chada Phisalaphong[2]

[1]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand.*
[2]*Government Pharmaceutical Organization, Bangkok, Thailand.*
E-mail: scnpm@mahidol.ac.th

Abstract

Introduction: Free radical is believed to play a major role in the pathogenesis of various disease including myocardial ischemia, neuronal cell injury and cancer. The most commonly formed free radicals in the biological system are superoxide anion radical, hydroxyl radical, peroxyl radical and nitric oxide.

Objective: This research was aimed to study free radical scavenging activity of curcumin, its demethoxy derivatives (demethoxycurcumin, Dmc and bisdemethoxycurcumin, Bdmc) and hydrogenated derivatives (tetrahydrocurcumin, THC, hexahydrocurcumin, HHC and octahydrocurcumin, OHC) on 1,1-diphenyl-2-picrylhydroxyl (DPPH), hydroxyl, superoxide anion, carbon-centered and nitric oxide radical.

Materials and methods: The free radical scavenging activity of curcumin and its demethoxy derivatives was studied by using electron spin resonance (ESR) spectroscopy and spin-trapping technique.

Results: The results demonstrated that all of the tested compounds, except Bdmc were more potent than the reference antioxidant, trolox, to scavenge DPPH radical. One mole of hydrogenated derivatives can scavenge about 4 moles, while curcumin and Dmc can scavenge about 3 moles of DPPH radical. The order of DPPH scavenging potency was in the decreasing order as follows: OHC>Dmc>=THC>=HHC> curcumin>trolox>>Bdmc. Curcumin and its derivatives showed moderate scavenging activities on nitric oxide and carbon-centered radical. About 0.5-1.0 moles and 0.1-0.5 moles of nitric oxide and carbon-centered radical, respectively, can be scavenged by one mole of the tested compounds. The decreasing order of nitric oxide scavenging potency was: curcumin>THC >=Dmc >HHC>=Bdmc>=OHC. The order of carbon-centered radical scavenging potency was decreased as follow: curcumin>THC>=Dmc >HHC>=Bdmc. For hydroxyl and superoxide anion radical, curcumin and its derivatives showed very low scavenging activity. One mole of the tested compounds could scavenge only 2-20 mmoles of these radicals.

Conclusion: Our results suggest that curcumin and its derivatives act as chain-breaking antioxidants rather than as direct scavengers.

Key words: antioxidant / curcumin / ESR spectroscopy

Introduction

Free radical is defined as molecule or molecular fragment containing one or more unpaired electrons in the atomic or molecular orbital. The most commonly formed free radicals in biological system are superoxide anion radical, hydroxyl radical, peroxyl radical and nitric oxide. Free radical production occurs continuously in all cells as a part of cellular function. However, excess free radical production originating from endogenous or exogenous sources can cause extensive damage to different types of molecules, including proteins, nucleic acids, lipids and DNA. This

process is involved in the pathogenesis of various diseases such as neurodegenerative disorder, cardiovascular disease and cancer.

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the principle yellow compound isolated from turmeric (Curcuma longa Linn), is widely used for the coloring of food. Natural turmeric extract contains curcuminoids. It comprises of curcumin (94%), demethoxycurcumin (Dmc) (6%) and bisdemethoxycurcumin (Bdmc) (0.3%). Because of its antioxidant, anti-inflammatory and anti-carcinogenic activity, curcumin is interested for its therapeutic purpose. In vitro, curcumin can protect RBC against H_2O_2-induced lysis and lipid peroxidation. In vivo, curcumin is shown to decrease the level of xanthine oxidase, reactive oxygen species and lipid peroxides while it causes an increase in the level of superoxide dismutase, catalase, GPx and GST.

Limited used of curcumin is caused by its poor bioavailability. In human, curcumin is metabolized into curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin (THC), hexahydrocurcumin (HHC) and octahydrocurcumin (OHC).

Electron spin resonance (ESR) spectroscopy with spin trapping technique is a unique technique for direct detection of transient free radical. The technique of spin trapping is that radical reacts with a spin trap to produce a ong-live free radicals which can be detected by ESR spectroscopy.

The aim of this study was to study the free radical scavenging activity of curcumin and its demethoxy derivatives (Dmc, Bdmc) and hydrogenated derivatives (THC, HHC and OHC) on DPPH (1,1-diphenyl-2-picrylhydrazyl) stable radical, hydroxyl radical, superoxide anion radical, carbon-centered radical and nitric oxide radical by spin trapping technique with electron spin resonance (ESR) spectroscopy.

Methods

Substrate: Curcumin, demethoxycurcumin (Dmc) and bisdemethoxycurcumin (Bdmc) were separated from curcuminoids (Government Pharmaceutical Organization, Bangkok, Thailand). Curcumin was converted to tetrahydrocurcumin (THC) by hydrogenation using palladium-carbon (Pd/C) as a catalyst. Hexahydrocurcumin (HHC) and octahydrocurcumin (OHC) were synthesized from THC by reduction with sodium borohydrie, and the products were confirmed using NMR spectra.

Free radical scavenging assay: Free radicals were generated from various methods. Hydrogen donating or radical scavenging ability of curcumin and its derivatives was evaluated by using a stable radical, DPPH. Hydroxyl radical was generated by Fenton reaction and superoxide anion radical ($O_2^{\cdot-}$) was generated by xanthine/hypoxathine system. DMPO was used as spin trapping agent for hydroxyl and superoxide anion radicals. Carbon-centered radical was generated from AAPH. AAPH were generated free radicals through spontaneous thermal decomposition, and 4-POBN was used as the trapping agent. Nitric oxide radical was generated from SNAP and [$(MGD)_2$-Fe^{2+}] was used as a spin trapping agent.

Data analysis:

The scavenging activity of curcumin and its derivatives was calculated as percentage of inhibition (% inhibition) by the following equation:

$$\%\text{inhibition} = \{100-[(\text{ESRarea(sample)} * 100)/\text{ESRarea(control)}]\}$$

The 50% inhibition concentration (IC_{50} value) was obtained from the plot between % inhibition and concentration of the antioxidant. Capacity value, the amount of scavenged free radical, was determined by using hydroxyl-TEMPO as a standard spin concentration. Total spin counts in the control reaction (without antioxidant) and in

the present of antioxidant were obtained from the graph between area of hydroxyl-TEMPO and concentration. Capacity value was calculated by the following equation:

Capacity value = [(Spin concentration of sample without antioxidant – Spin concentration of sample with antioxidant)] /Concentration of antioxidant)

Since DPPH itself is a radical, capacity value was calculated directly from its area.

Results

Free radical scavenging capacity value of curcumin and its derivatives is shown in Table 1.

Table 1: Comparison of capacity value of curcumin and its derivatives determined by various methods.

Substrate	Capacity values (mole/mole antioxidant)				
	H-donor	C·	NO	$O_2^{\cdot}(10^{-3})$	$HO^{\cdot}(10^{-3})$
Curcumin	3.37	0.54	0.99	18.21	7.09
Demethoxycurcumin	3.13	0.25	0.67	8.22	14.05
Bisdemethoxycurcumin	1.73	0.19	0.54	6.69	4.04
Tetrahydrocurcumin	4.10	0.30	0.86	9.14	18.70
Hexahydrocurcumin	3.69	0.14	0.56	7.71	2.45
Octahydrocurcumin	3.82	no effect	0.48	12.5	5.86

Values are expressed as mean±SD (n=3) of 5 experiments.

Discussion

Curcumin and its derivatives showed the highest scavenging activity on DPPH radical. Hydrogenated derivatives are more potent than curcumin, which one mole of hydrogenated derivatives can scavenge about 4 moles of DPPH-radical. Curcumin and its derivatives, show moderate scavenging activities on nitric oxide and carbon-centered radical. Except for OHC, about 0.1-1.0 moles of nitric oxide and carbon-centered radical can be scavenged by curcumin and its derivatives. Curcumin and its derivatives show low scavenging activities for hydroxyl, superoxide anion. One mole of the tested compounds could scavenge only 2-20 mmole of those radicals. THC and Dmc are the most potent for inhibition of hydroxyl radicals while curcumin is the most potent for inhibition of superoxide anion radicals.

Conclusion

Because of their effective scavenging property for of DPPH and carbon-centered radicals, it can be concluded that curcumin and its derivatives act as chain-breaking antioxidants rather than as direct radical scavengers.

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P09 Atorvastatin Attenuates LPS-Stimulated Cell via Toll–like Receptor 4

Praveen Chansrichavala[1], Piyamitr Sritara[2], Udom Chantharaksri[1], Sansanee C. Chaiyaroj[3],

[1]*Department of Pharmacology, Faculty of Science, Mahidol University,*
[2] *Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University,*
[3]*Department of Microbiology, Faculty of Science, Mahidol University*
E-mail: scscy@mahidol.ac.th

Abstract

Introduction: In the last decades more substantial insights to atherosclerosis have pointed out a pivotal role of innate immunity in the initiation and development of the disease. With clearer insight comes also the understanding and evidence suggesting that statins, HMG-CoA reductase inhibitors, have other beneficial effects that are lipid-lowering independent. One of the main innate components that have spurred a great interest in the atherosclerosis field is Toll-like receptors, TLRs. TLRs are central recognition and signaling receptors in host defense against pathogens or danger signals. In the case of atherosclerosis, these beings heat shock proteins 65/60 and oxidized LDL (oxLDL). Evidence has implicated that oxLDL can upregulate TLR4. Although it has been established that TLR4 plays a role in the development of atherosclerosis but a study has yet to show the direct interaction of TLR4 and statin.
Objective: In this study we aimed to study the anti-inflammatory effect of atorvastatin *in vitro* employing Ba cell lines transfection system (kind gifts from Prof. K. Miyake, Tokyo University, Japan).
Materials and methods: To study the anti-inflammatory effect of atorvastatin via Toll-like receptor 4 we looked at their NF-κB activities via luciferase measurement.
Results: Our results showed that atorvastatin can attenuate LPS-stimulated pro-B cell lines through NF-κB dose dependently and mevalonate can reverse atorvastatin inhibitory effect. Since mevalonate could reverse the inhibitory effect of atorvastatin, we further explored the relationship between mevalonate and TLR4. However, we found that mevalonate does not exert its effect by direct binding to TLR4 suggesting that TLR4 is not directly involved in the mechanism of action of mevalonate.
Conclusion: The results implicated that atorvastatin, a lipid-lowering drug employed in the treatment of hypercholesterolemia and atherosclerosis, has immunomodulatory role which does not involve their lipid lowering effect.
Key words: atorvastatin, NF-κB , Toll–like receptor 4

Introduction

Atherosclerosis begins as an inflammatory immunological disease. Atherosclerosis lesions are the result of a series of highly specific cellular and molecular responses to various endogenous risk factors and antigenic such as oxidized LDL (oxLDL) (1), heat shock protein 65/60 (1, 2), β_2-glycoprotein Ib (1) These stimuli lead to a sustained inflammatory responses resulting in the progression and destabilization of atherosclerotic plaques. Recently, it has been suggested that cells of the innate and adaptive immunity play a central role in atherosclerosis (1).
Statins inhibit HMG-CoA reductase, which is the rate-limiting step of cholesterol biosynthesis. Previous studies have pointed out statins' crucial beneficial

effects which are lipid-lowering independent suggesting that statins have immunomodulatory role. Kwak et al. showed that statins act as direct inhibitors on induction of major histocompatibility class II (MHC-II) expression by interferon-γ (IFN-γ), thus as repressors of MHC-II-mediated T cell activation (3). This unexpected effect provides a scientific rationale for using statins as immunosuppressors, not only in organ transplantation but in numerous other pathologies as well, for example, atherosclerosis (3),(4). Atorvastatin, a third generation of statin, has been shown to reduce proinflammatory markers (tumor necrosis factor [TNF], IL-1, and IL-6) as well as soluble intercellular adhesion molecule-1 (sICAM-1), and C-reactive protein (CRP) in hypercholesterolemic patients (5).

One of the innate components that are thought to play a role in the development of atherosclerosis is Toll-like receptors (TLRs). TLRs are central recognition and signaling receptors in host defense against pathogens or danger signals (6). In the case of atherosclerosis, these beings heat shock proteins 65/60 and oxidized LDL (oxLDL). Evidences have implicated that oxLDL can upregulate TLR4 (7) and it has been proposed that oxLDL may act as ligand for TLR4 (8). Although it has been established that TLR4 plays a role in the development of atherosclerosis, no study to date has shown the direct interaction of TLR4 and statin.

In this study we investigated the role of anti-inflammatory effect of atorvastatin in vitro employing Ba cell lines transfection system.

Materials and Methods

Cell culture and stimulation

Approximately 10^5 cells were seeded into 96-well plate. Cells were stimulated with 1μg/mL LPS for 24 hours. Cells were then treated with atorvastatin at various concentrations (0.1, 1, 10 μM) and incubated for different periods (0, 12, 24, 48 hr). In some experiments, cells were treated with atorvastatin and 100 μM mevalonate (Sigma, USA) simultaneously.

To study the interaction between mevalonate and TLR4, cells were stimulated with various concentrations (1, 10, 100 μM) of mevalonate. LPS stimulated cells were used as positive controls.

Luciferase Assay

Briefly, cells were flushed and 100 μL of cells were removed and transferred to 96-well white plate. Then 100 μL of Steady Glo® substrate was added and incubated in dark for 5 minutes. The luciferase activities were quantitated using an Automated Microplate Reader machine.

Results

Atorvastatin attenuates NF-κB activities in LPS-stimulated cells

Cells were stimulated with LPS for 24 hours then treated with atorvastatin at various concentrations (0.1, 1, 10 μM) for different periods of time (0, 12, 24, 48 hr). We found that at 10 μM atorvastatin significantly inhibited NF-κB activities after 24 hr incubation period, while longer incubation period was required for lower concentrations.

Mevalonate reverses atorvastatin inhibitory effect in LPS-stimulated cells

LPS stimulated cells were treated with atorvastatin and mevalonate simultaneously. The NF-κB activities were measured and our results demonstrated that mevalonate could significantly reverse inhibitory effect of atorvastatin on NF-κB activities.

Mevalonate does not bind to TLR4

The reversal effect of mevalonate led to further exploration of the interaction between mevalonate and TLR4. Cells were treated with various concentrations (1, 10, 100 μM) of mevalonate and incubated for different periods of time (0, 12, 24 hr). The luciferase activities were then measured. The results showed that mevalonate did not bind to TLR4 as no significant difference in NF-κB activities were seen when compared with LPS-stimulated cells.

Discussion

New insights to atherosclerosis have pointed out an important role of innate immunity in the initiation of the disease. Previous studies (3, 4) have implicated that statins have immunomodulatory role that is lipid-lowering independent. In this study we investigated the role of atorvastatin on NF-κB activities in TLR4 transfected cell line system.Atorvastatin could attenuate LPS-stimulated pro B-cells dosedependently while mevalonate, which is a downstream precursor in the cholesterol biosynthesis pathway could reverse this inhibitory effect of atorvastatin. However, mevalonate reversal effect is not through the direct binding to TLR4 suggesting that mevalonate employs other pathway in exerting its effect. The pleiotropic nature of statins is impressive and our data have demonstrated one such effect namely, anti-inflammation.Together, the results implicate that atorvastatin anti-inflammatory effect could be beneficial in patients with coronary heart diseases.

Acknowledgment

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P10 Neuromuscular Blocking Effect of Semipurified Fractions of King Cobra (*Ophiophagus Hannah*) Venom

Jureeporn Noiphrom[1], Orawan Khow[2], Sopit Thamaree[3]

[1]*Inter-departmental Program in Pharmacology, Graduate school, Chulalongkorn University*
[2]*Queen Saovabha Memorial Institute, Thai Red Cross Society*
[3]*Department Pharmacology, Faculty of Medicine, Chulalongkorn University University*

Abstract

Introduction: Human envenomed by king cobra, *Ophiophagus hannah*, developed muscle paralysis. The neuromuscular blocking effect of king cobra venom may be of interest for developing a therapeutic agent.

Objective: To investigate and screen the neuromuscular effect of king cobra crude venom and its fractions and study the mechanism of and site of action.

Materials and methods: The semipurified fractions of king cobra venom possessing the neuromuscular blocking action were tested on the isolated mouse's phrenic nerve-diaphragm.

Results: The crude venom showed dose-dependent neuromuscular blocking effect. The crude venom was fractionated using ion exchange chromatography and seven fractions (fractions No.1 – No.7) were obtained. The first fraction was further fractionated to obtain 3 fractions (fractions No.1.1 – No.1.3). The time (min) taken to produce 50% inhibition of the twitch tension by the crude venom (80 μg/ml), fractions No.1 (30.5 μg/ml); No.1.1 (7.3 μg/ml) and No.1.2 (16.6 μg/ml) were 4.2 ± 0.1, 4.6 ± 0.2, 42.8 ± 2.7 and 4.3 ± 0.3 min, respectively. Fraction No.1.2 showed the highest potency of neuromuscular blocking effect.

Conclusion: The result suggested that fraction No.1.2 should be selected for further fractionation and purification to obtain a pure compound and investigated for its mechanism of neuromuscular blocking action.

Keywords: *Ophiophagus hannah*, neuromuscular, neurotoxin, phrenic nerve-diaphragm

Introduction

King cobra, *Ophiophagus hannah*, which belongs to the Elapid family, is believed to be the world's largest poisonous snake and injects a large amount of venom once. Because of its venomous neuromuscular effect, humans envenomed by *O. hannah* develop muscle paralysis and the death of victims is caused by respiratory paralysis. Therapeutic agents have been developed from their the neuromuscular effect, for example, tubocurarine, the first muscle relaxant to be introduced to anaesthetic practice and botulinum toxin being used to treat cases of blephrospasm and similar syndromes associated with overactivity of skeletal muscle (1). Neuromuscular blocking effect investigation of king cobra venom including its fractions for drug development is interesting. The objectives of this study can be divided into two main points: 1) to screen the neuromuscular effect of king cobra crude venom and its fractions and to select the best one, and 2) to study the mechanism of and site of action, pre- or postsynaptic, and whether it affects the direct muscle stimulation.

Methods

1. Isolated mouse's phrenic nerve-diaphragm preparation

The phrenic nerve-diaphragm preparations (2) were isolated from 30-40 g male Swiss Albino mice anesthetized with 100% CO_2, and mounted under the ~0.8 g resting tension in the 25 ml organ bath containing Krebs solution of the following composition (mM): NaCl (118); KCl (4.8); KH_2PO_4 (1.2); $CaCl_2$ (2.5); $NaHCO_3$ (25); $MgSO_4$ (2.4) and D-glucose (11). The solution was maintained at 34°C and aerated with 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate for about 40-60 min with solution changes at every 20-30 min.

2. Neuromuscular blocking effect of the crude venom, and its fractions (3).

For indirect stimulation, the phrenic nerve was electrically stimulated with supramaximal (4x thresholds) pulses of 0.2 ms duration at a frequency of 0.1 Hz. The twitch tension was recorded and the time taken to maintain the stable tension was observed (control, n = 10). The preparations were allowed to stabilize for 10 min before the addition of king cobra venom or its fractions. The crude venom and its fractions were tested (n = 5). The final concentrations of crude venom were 5, 10, 20, 40, 80 and 160 μg/ml. The concentrations of tested fractions were calculated according to % yield of each fraction. The time (min) taken to produce 50% inhibition of the twitch tension was determined.

Results

The control preparations could maintain the stable twitch tension at >95% within 60 minutes. The crude venom caused a dose-dependent neuromuscular blockade and the time taken to produce 50% inhibition was shown in Table 1 and Figure 1. The crude venom at the concentration of 80 μg/ml was considered to be the lowest concentration that produced the highest neuromuscular blockade.

The tested concentrations of fractions are shown in Table 2. The fractions No.1, No.1.1 and No.1.2 caused neuromuscular blockade whereas the others could not reach 50% inhibition within 60 minutes. The data are shown in Table 2 and Figure 2. The result showed that fraction No.1.2 was more effective than fraction No.1.1.

Discussion

Neurotoxins, pre- or postsynaptic neurotoxin, are the main toxic proteins from snake venom which block neuromuscular transmission. The neuromuscular effect can be investigated on the isolated mouse's phrenic nerve-diaphragm preparations. For the control preparations, the twitch tension was allowed to stabilize >95% in order to assure that other factors could hardly affect the tension after venom or fractions adding. The tests were done within 60 minutes which could maintain the stable tension at >95%. The neuromuscular blockade caused by the crude venom was dependent on its concentrations. The concentration of 80 μg/ml of the crude venom represented the lowest concentration that produced the highest neuromuscular blockade (Figure 1). The neuromuscular blocking effect of the crude venom (80 μg/ml), the fraction No.1 (30.5 μg/ml) and the fraction No.1.2 (16.6 μg/ml) gave nearly the same time (min) taken to produce 50% inhibition of the twitch tension (4.2 $\pm$ 0.1, 4.6 $\pm$ 0.2 and 4.3 $\pm$ 0.3 min, respectively). The result showed a coincidence of their twitch tension (Figure 2). This means that the effective fraction was more potent than the crude venom at the equal concentration. The fraction No.1.1 was slightly effective, took 42.8 $\pm$ 2.7 min to cause 50% inhibition of the twitch tension. It may be possible that there is overlapping between the fraction No.1.1 and No.1.2. Other

fractions might play some roles in neuromuscular effect but much less. The fraction No.1.2 will be fractionated later for more purification.

Conclusion

In this study, the neuromuscular blocking effect of the crude venom was dose-dependent. The minimum concentration needed for maximum neuromuscular blockade was 80 μg/ml. The effective fraction was more potent than the crude venom at an equal concentration. The fraction No.1.2 was selected to be further fractionated and more purified. The mechanism of neuromuscular blocking action of the pure compound should be investigated.

Acknowledgement

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Table 1 Time (min) taken to cause 50% inhibition by the crude king cobra venom

Concentration (μg/ml)	5	10	20	40	80	160
Time taken (min) to cause 50% inhibition	23.4 ± 0.9	19.7 ± 0.5	9.1 ± 0.4	6.9 ± 0.3	4.2 ± 0.1	4.0 ± 0.1

Crude king cobra venom at the concentration of 160 μg/ml, the highest concentration studied, took the least time to cause the 50% inhibition of the twitch tension. Therefore, the concentration of 80 μg/ml which produced nearly the same result was selected to be used to calculate the concentration of the fractions. The data were expressed as mean ± S.E.M (n = 5).

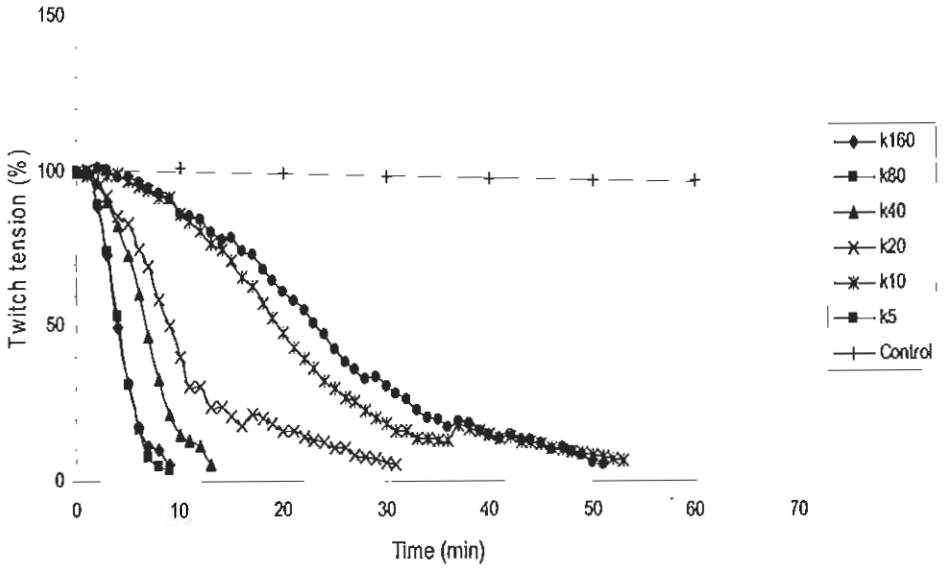

Figure 1 Neuromuscular blockade induced by the crude king cobra venom on the isolated mouse's phrenic nerve-diaphragm preparation.

Each point represents the average from five experiments of the crude venom at the concentrations of 5, 10, 20, 40, 80 and 160 μg/ml (k5, k10, k20, k40, k80 and k160, respectively). The control data were collected from ten experiments.

Table 2 The tested concentrations of the first and the second fractions and its neuromuscular effect

Fraction No.	Yield (%)	Concentration (μg/ml)	Time taken to caused 50% inhibition (min)	Twitch tension at the 50th minute after fractions added (%)
1	38.1	30.5	4.6 ± 0.2	-
2	10.4	8.3	-	83.4 ± 2.1
3	9.3	7.4	-	70.7 ± 1.9
4	11.3	9.0	-	67.5 ± 4.5
5	11.3	9.0	-	90.1 ± 2.9
6	4.1	3.3	-	84.3 ± 2.9
7	5.4	4.3	-	83.5 ± 2.7
1.1	23.9	7.3	42.8 ± 2.7	-
1.2	54.4	16.6	4.3 ± 0.3	-
1.3	29.2	8.9	-	70.5 ± 3.1

The fractions No.1 – No.7 were obtained from the first fractionation of the crude venom by ion exchange chromatography. The fractions No.1.1 – No.1.3 were obtained from the second fractionation of the fraction No.1. Only the fraction No.1, No.1.1 and No.1.2 caused neuromuscular blockade, the data were expressed as mean±S.E.M (n = 5).

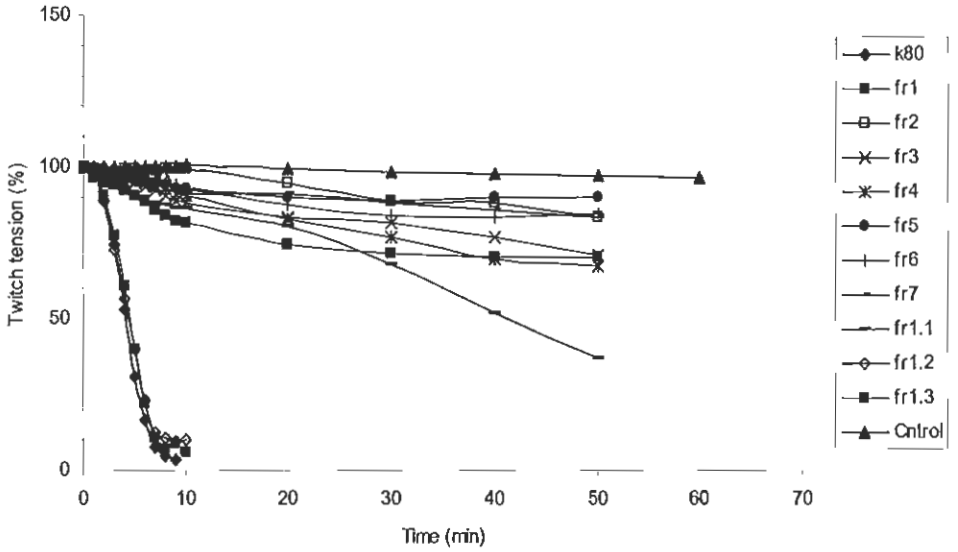

Figure 2 Neuromuscular blockade induced by the crude king cobra venom and the fractions on the isolated mouse's phrenic nerve-diaphragm preparation.

Each point represents the average from five experiments of the crude venom at the concentration of 80 μg/ml (k80), the fractions No1 – No.7 and the fractions No1.1 – No.1.3 at the concentrations shown in Table 1. The control data were collected from ten experiments.

P11 Tetrahydrobiopterin induced dopaminergic cell death via apoptotic mechanisms and tyrosine hydroxylase activation

Vasutakarn Chongthammakun[1,3], Sukumal Chongthammakun[2,3], Yupin Sanvarinda[1,3]

Department of Pharmacology[1], Department of Anatomy[2] and Center for Neuroscience[3], Faculty of Science, Mahidol University
E-mail: g4637305@student.mahidol.ac.th

Abstract

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in substantia nigra. Increasing recent evidence has focused on the role of endogenous molecules in the progress of dopaminergic cell death.

Objective: To demonstrate the toxic effect of tetrahydrobiopterin (BH4), an obligatory cofactor for dopamine (DA) synthesis, along with its mechanisms, on dopaminergic neurons

Materials and methods: The toxic effect of tetrahydrobiopterin (BH4) on dopaminergic neurons was studied using human SH-SY5Y neuroblastoma cell line.

Results: BH4 treatment at varying concentrations induced neuronal death in a dose-dependent manner, as determined by lactate dehydrogenase (LDH) assay. Apoptotic cell death upon BH4 incubation was characterized by the increased expression of apoptotic markers, Bax/Bcl-2 ratio and cleaved caspase-3. BH4 also stimulated phosphorylation of tyrosine hydroxylase (TH), the rate-limiting enzyme for DA synthesis. Consistent with the finding on TH activation, DA release was slightly increased upon BH4 exposure.

Conclusion: These data suggested that the toxicity of extracellular BH4 on dopaminergic neurons may involve the activation of TH enzyme and subsequent increases in DA content and release. Additionally, the mode of BH4-induced dopaminergic cell death was indeed apoptosis.

Key words: tetrahydrobiopterin, Parkinson's disease, tyrosine hydroxylase, dopamine, apoptosis

Introduction

Up to date, the etiology of Parkinson's disease (PD) remains enigmatic, with many hypotheses being raised in effort to elucidate the cause and molecular mechanisms underlying PD. Since it is well known that dopaminergic neurons are more vulnerable than any other neurons, it is likely to postulate that some specific endogenous molecules confined to this type of neurons are capable of rendering toxicity, ensuing dopaminergic cell death. The molecules that are likely candidates for this cause include dopamine (DA), iron, tyrosine hydroxylase (TH) enzyme, and tetrahydrobiopterin (BH4).

Focusing on BH4, although not much has been learned about its detrimental effects on dopaminergic neurons, increasing evidence has introduced the role of extracellular BH4 as the selective contributor of dopaminergic cell damage in both *in vivo* and *in vitro* models (1,2). However, the exact mechanisms in which dopaminergic neurons undergo cell death upon BH4 exposure are not yet certain.

As DA is known to exert toxicity through its DA-quinone derivatives produced during DA auto-oxidation (3) and TH is renowned for its ability to generate

reactive oxygen species (ROS) and also DA (4), therefore, it is possible that DA and TH may all be implicated in BH4 toxicity. Thus, the objective of this study is to investigate the toxic effect of extracellular BH4 on dopaminergic neurons and its underlying molecular mechanisms.

Materials and Methods

Cell cultures

Human SH-SY5Y neuroblastoma cell line were grown in a 1:1 mixture of Eagle's Minimal Essential Medium (MEM) and Nutrient Mixture Ham's F-12 medium, supplemented with 10% fetal bovine serum. At confluence, the cells were harvested and seeded onto culture plates or dishes for further experiments.

Lactate dehydrogenase (LDH) assay

LDH release into the medium after treatment with BH4 and/or other chemicals was detected by using an LDH assay kit (Sigma) according to the manufacturer's instructions. The results of LDH values were expressed as percentage of untreated control.

Western blot analysis

After exposure to BH4 for certain different times, the cells were lysed and total proteins were collected. Equal amounts of proteins were loaded on to SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated in blocking buffer, followed by incubation with primary antibody and subsequent corresponding secondary antibody. Detection was performed by using ECL reagents and exposed onto films.

[H^3]Dopamine release

The cells were loaded with [H^3] DA and were exposed to BH4 for various intervals. Scintillation fluid was then added to the removed medium and the radioactivity was measured. Data were expressed as percentage release as compared to untreated control at the equivalent time point.

Statistical analysis

All data are expressed as means ± SEM. Differences among groups were evaluated by one-way ANOVA followed by Student-Newman-Keuls multiple comparisons tests. Statistical significance was considered when $p<0.05$.

Results

BH4 stimulated neuronal cell damage in a dose-dependent manner

BH4 exposure to SH-SY5Y cells for 24 h showed dose-dependent increased in LDH release. The LDH values were 108.4 ± 2.2%, 111.4 ± 1.7%, 169.1 ± 5.0% ($p<0.001$ *vs.* control), and 217.6 ± 9.9% ($p<0.001$ *vs.* control) for 50 μM, 100 μM, 200 μM and 400 μM BH4-treated groups, respectively, which suggested that the toxic effect of BH4 on dopaminergic neurons was mediated in a dose-dependent manner.

Involvement of apoptotic pathways in BH4-induced cell death

Western blot analysis of SH-SY5Y cells treated with BH4 for 12 and 24 h demonstrated changes in Bax and Bcl-2 immunoreactivity, in which the expression of Bax gradually increased from 12 to 24 h while that of Bcl-2 slightly decreased. β-actin was used as internal control. The Bax/Bcl-2 expression ratio was also determined. This ratio has been shown to determine the cell's susceptibility to apoptotic stimuli and has been widely used as an index for apoptosis. The ratio of Bax/Bcl-2 band intensity was elevated to 203.8 ± 57.9% of control at 12 h and to 193.0 ± 25.0% of control at 24 h after BH4 exposure. The increase in Bax/Bcl-2 ratio indicated an imbalance between the pro- and anti- apoptotic regulators of the mitochondria, shifting the cells toward programmed cell death.

Caspase-3 is one of the key mediators of apoptosis responsible for the proteolytic cleavage of vital proteins of the cells. From western blot analysis, untreated control showed strong expression of the inactive pro-caspase-3, which was decreased when BH4 was present for 12 and 24 h. In contrast, the immunoreactivity of the active cleaved caspase-3 was nearly absent in control group, but was evident in both the groups treated with BH4. Therefore, this experiment demonstrated that BH4 exposure resulted in the activation of caspase-3, leading to apoptosis.

BH4-stimulated TH phosphorylation

Previous evidence has shown that phosphorylation of TH provides a greater affinity for its cofactor, resulting in an increased rate of DA synthesis (5). To investigate whether BH4-induced dopaminergic cell death was mediated via TH activation, BH4-treated SH-SY5Y cells for various time periods were detected for phosphorylated form of TH enzyme by western blot analysis.

Initial rapid increase in TH phosphorylation from 0 to 30 min was observed and maximal phosphorylation was recognized at 30 min, where it finally recovered to baseline within 24 h. β-actin was used as internal control. These results indicated that the enhanced activity of TH following BH4 incubation may possibly lead to subsequent increase in DA synthesis and DA content, which may help contribute to dopaminergic neuronal damage after exposure to BH4.

The effect of exogenous BH4 on spontaneous DA release

To further clarify whether BH4 had an effect on DA release, the amount of [H^3] DA in the medium was quantified after BH4 treatment. The quantity of [H^3]DA released from the cells at 15, 30, 45, and 60 min were 108.7 ± 4.3, 120.0 ± 10.4, 107.7 ± 2.4, and 104.4 ± 0.6%, respectively, as compared to untreated control at the same time interval, suggesting a tendency for spontaneous DA-releasing action of BH4.

Discussion

The role of BH4 in the induction of dopaminergic cell death was characterized in this study. From the results, treatment of SH-SY5Y cells with exogenous BH4 resulted in neuronal cell death in a dose-dependent fashion, and furthermore, apoptotic mechanisms have been shown to be the mode of cell death from BH4 exposure. It is well known that apoptotic pathways usually predominate in the cell death paradigm of dopaminergic neurons as they normally demonstrate apoptotic features upon exposure to neurotoxins (6). Our data from this study on changes in protein expression of the apoptotic markers following BH4 exposure have supported this speculation. In addition, it may be interpreted from the results that BH4 induces apoptosis in SH-SY5Y cells at least via the intrinsic mechanisms that involve the changes in mitochondrial permeability; however, the activation of apoptosis via the extrinsic pathway, which involves the binding of death receptor to its ligand, can not yet be excluded.

The other findings of this study involve the mechanisms of BH4-induced cell death through the enhancement of DA toxicity. As DA is long known to induce oxidative stress via nonenzymatic auto-oxidation as well as enzymatic degradation by monoamine oxidase (MAO) (3), an agent that would exacerbate these phenomena should promote damage to dopaminergic neurons. Thus, the TH enzyme that was activated following BH4 incubation in the present study may result in increased DA level and subsequent toxicity. Supported by a number of evidence, the activity of the *in vivo* and *in vitro* TH was found to be elevated following exogenous BH4 administration (7, 8). However, this is the first study to provide evidence that BH4 activates TH enzyme via phosphorylation.

It is well known that the production and release of catecholamines are closely linked. This study demonstrated that BH4 had an effect on DA release which may be the result of the increased DA synthesis from the activated TH. The released DA in the extracellular space would certainly exacerbate the toxic effect of BH4. However, more tests are needed in order to exclude the possibility that BH4 directly triggers DA release independent of DA synthesis.

Conclusion

Based on the results of this study, it is proposed that BH4, a necessary cofactor for DA synthesis which is present exclusively in monoaminergic neurons, can contribute to dopaminergic cell damage when present extracellularly. The mechanisms for dopaminergic cell death upon contact with extracellular BH4 are also proposed; thereby suggesting that BH4 may exist as one of the potential contributors in dopaminergic neuronal cell death in PD as very small amounts of ROS and DA quinone radicals produced or stimulated by BH4 over a long period of time could be one of the factors responsible for the cause of PD.

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P12 Preliminary Study on Sedative Effect of Guava (*Psidium guajava L.*) Leaf Oil

Amonrat Khayungarnnawee, Wipaporn Phatvet, Tuanta Sematong, Sirinan Thubthimthed and Taweesak Suntorntanasat

Pharmaceutical and Natural Products Department, Thailand Institute of Scientific and Technological Research, Technopolis, Klong 5, Klong Luang, Pathumthani
E-mail: amonratkh@yahoo.com

Abstract
The preliminary pharmacological study on the sedative effect of guava (*Psidium guajava L.*) leaf oil *via* inhalation route in mice was carried out by locomotor activity cage. This cage made of a cubicle of clear Perspex (54x50 cm^2, 37 cm high, Ugo Basile 7431). It could automatically monitor the horizontal or vertical movements of the animals by counting the number of time, each animal crossed the infrared beams. Lavender oil was used as standard oil (positive control). It showed that the guava leaf oil exhibited a reduction in motility in mice (57.15%) as lavender oil which is widely known to have a sedative activity (29.65%).
Keywords: *Psidium guajava L.*, guava leaf oil, sedative effect, locomotor activity

Introduction

Psidium guajava L. (Myrtaceae) is commonly known as guava. Medicinal properties of guava leaf include antidiarrheal, antispasmodic, anti-inflammatory, anticough and sedative effects. In this paper, we conducted the experiment to study the CNS depressive effect of guava leaf oil upon inhalation in mice to support its use as aromatherapy products.

Materials and methods

Plant materials and extraction

The fresh leaves of guava were extracted by hydrodistillation for 5 hours. The oil was pale yellow and the yield was 0.2% (v/w). It was analyzed by capillary GC and GC/MS. Limonene was a major chemical composition.

Animals

Male IRC mice of body weight between 25-30 g were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. The animals were housed in animal care facility at Thailand Institute of Scientific and Technological Research for 1 week before experimentation.

Method

Male IRC mice were divided into 3 groups (6 mice/group). The first group was used for testing guava leaf oil (5% v/v) and the other two groups with lavender oil (5% v/v) and distilled water as a positive control and control group, respectively. The test samples were dosed to the animals *via* inhalation route. The sedative effect was studied using locomotor activity cage. This cage was made of a cubicle of clear Perspex (54x50 cm^2, 37 cm high, Ugo Basile 7431). It could automatically monitor the horizontal or vertical movements of the animals by counting the number of times, each animal crossed the infrared beams. Mice were measured for their locomotor activity before and after inhalating the test samples.

Result and discussion

Guava leaf oil exhibited a reduction in motility as does the lavender oil which is already known as an agent having a sedative effect (Table 1).

Table1. Percentage of animal activity after essential oil inhalation

Group	No.	% Inhibition
Control	6	No difference
Lavender oil	6	29.65
Guava leaf oil	6	57.15

The sedative effect of guava (*Psidium guajava L.*) leaf oil *via* inhalation route in mice was determined by locomotor activity cage as a preliminary study. Lavender oil, already known oil having a potent sedative effect was used as a positive control. The data showed that the guava leaf oil possessed sedative effect, compared to the oil.

Guava leaf oil has sedative effect on CNS and its mechanism mechanism of action should be further studied and clarified.

Acknowledgment

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P13 Toxicological Evaluation of Commercial Thai Herbal Preparations

Nanthiya Rattanakhot[1], Patoomrat Toojinda[2], Panya Temjarean[3], Kampon Sriwatanakul[4], and Auratai Aramphongphan[1, 4]

[1] *Toxicology graduate program, Faculty of science, Mahidol University*
[2] *Department of Chemistry, Faculty of science, Mahidol University*
[3] *Department of Pathobiology, Faculty of science, Mahidol University*
[4] *Department of Pharmacology, Faculty of science, Mahidol University*
E-mail: scaap@mahidol.ac.th

Abstract

Introduction: Herbal medicines have been gaining popularity worldwide. Conceptually, herbs are usually considers non-toxic by the general public due to their natural origin. However, the consumption of herbs is well known to be capable of producing adverse health effects.

Objective: In this study, six commercially available herbal preparations, Ya-ayu-wattana (Thai herbal "elixir"), some herbal weight loss products, Black pepper herbal drug (Piper nigrum), Ka-min-chan (Curcuma longa), Wan-Chak-Mod-Luk herbal drug (Curcuma xanthorrhiza), and essential oil of Plai (Zingiber cassumunar), were selected for the acute oral toxicity and mutagenicity test.

Materials and methods: Five herbal preparations, Ya-Ayu-wattana, herbal weight loss product, Black pepper herbal drug, Kha-min-chan herbal drug, and Wan-chak-mod-luk herbal drug were extracted with 95% ethanol and dried by lyophilizer. The essential oil of Plai was extracted by steam distillation. Acute oral toxicity was tested in rats and mutagenicity was tested using bacterial reverse mutation test system.

Results: Acute toxicity studies shown that the essential oil of Plai had high toxic effects while other herbal preparations shown no sign of toxicity. The toxicity signs were salivation, weakness, decreased muscle tone, involuntary urination, hypersecretion, and dypnea as well as death at the concentrations of 1500 mg/kg BW in a male rat and 750 mg/kg BW in female rats. The histopathological examination of the dead animals after essential oil of Plai administration indicated mild to severe congestion, acute interstitial pneumonitis, intraalveolar hemorrhage, and pulmonary edema. In the liver, mild to moderate liver cell congestion, swelling of hepatocytes, and microvesicular fatty change had occurred. In the kidney, mild to moderate congestion and focal cloudy swelling of tubules were observed. The severity of toxicity depended on the dose. Essential oil of Plai caused reduction in body weight and food consumption, the results shown significant decrease in the body weight and food consumption when compared to the control group ($p<0.05$). Six herbal extracts were further study for mutagenic property by bacterium test system. And the results of Ames' test exhibited no mutagenic effect of the six herbal extracts tested in the absence and presence of S9 fraction. However, Plai extracted possessed dose response to Salmonella typhymurium TA98 with S9 mix test system.

Conclusion: The results from this study suggested that the essential oil of Plai is not appropriate for use as an oral herbal medicine. The evaluation of the toxicity of these herbal preparations should be clarified.

Keywords: *Thai herbal preparations / Acute toxicity study/ Mutagenicity / Ames' test*

Introduction: Herbal medicines have been gaining popularity worldwide. An estimated one third of adults in the Western world use alternative therapies, including herbal treatments. The herbs may be used either in their primary forms or combined into mixtures. In contrast to pharmaceutical compounds, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines. In Thailand, this belief still pervades because of the limited information on toxicity in Thai herbs. Therefore, this research tries to investigate the toxicity of commonly used herbs by acute oral toxicity study and Ames'test to assess their mutagenic activities.

Materials and methods:

1. Acute oral toxicity study:

1.1 The range-finding study: Each test material was given as a single dose by gavages technique to rats. Control group received the same volume of 10% dimethylsulfoxide (DMSO) in corn oil or corn oil. One male and one female rat were fasted 16 hr before treated with the test chemical at 500, 1000, 2000, 3000, or 5000 mg/kg BW. Animals were observed for 7 days, for the onset of toxic signs and symptoms, and time of death. Tissues and organs were collected and fixed in 10% buffer formalin for histological examination.

1.2 Acute toxicity test: the herbal product that caused clinical symptoms and mortalities at the dose below 2000 mg/kg BW was further investigated in large number of animals at various doses ranging from 500 to 2000 mg/kg BW.

2. Ames' test: The bacterial reverse mutation test (Ames'test) was used to detect genotoxicity properties of the crude extracts of Plai, Wan-chak-mod-luk, Cha-min-chan, Black pepper, herbal weight loss products, and Ya-ayu-wattana. The crude extracts of these herbs were dissolved by DMSO into concentrations. The concentrations of Plai were 1, 10, 20, 25, 50 µg/plate. The concentrations of Wan-chak- mod-luk and Cha-min-chan were 1, 10, 20, 50, 100 µg/plate. The concentrations of Black pepper were 1, 10, 25, 50, 100 µg/plate. The concentrations of herbal weight loss products, and Ya-ayu-wattana were 1, 10, 20, 50, 100, 500 µg/plate. The 0.1 ml. of crude extract of herb tested, 0.1 ml of tester strain, and 0.5 ml of buffer or S9 fraction were mixed and incubated at 37°C, 20 minutes before pouring into minimal glucose agar plate for incubation at 37°C for 48 hr. Finally, the revertant colonies were counted to compare with negative and positive controls.

Results and Discussion

The range-finding study found that the crude extract of Plai, Wan-chak-mod-luk, Cha-min-chan, Black pepper, herbal weight loss product, and Ya-ayu-wattana at a single oral dose of 500, 1000, 2000, 3000, or 5000 mg/kg BW did not cause death, or clinical symptoms in both male and female rats. Acute toxicity testing showed that the essential oil of Plai at a single dose of 2000, 3000 or 5000 mg/kgBW caused fatal, while the dose of 500 mg/kg caused no death and no toxicity symptoms in both sexes. The mainly target organs of toxicity were lung, liver and kidney. The toxic signs and symptoms were salivation, weakness, decreased of muscle tone, involuntary urination, dypnea, and death. The dose of 1000 mg/kg BW caused death in female rats, suggesting that the female rats are more susceptible to toxicity of Plai. The reductions in body weight and food consumption were observed in the Plai treated groups (2000 mg/kg) as compare to the control.

No mutagenic effect was found in any of the herb extracts by the Ames' test. However, Plai extracts showed a dose-response effect in Salmonella Typhimurium TA 98 with S9 fraction. Since the Ames' test has a particular limitation by the

solubility of test chemical. Therefore, further studies should be done to assess the genotoxicity by using mammalian cells culture or other models for chromosome aberration to determine other possible genotoxic effects of these herbs before doing a long-term study in whole animals.

Conclusion

Essential oil of plai has long been used via inhalation or massage as spa products or externally as pain relief cream. The toxic effect, if there is any, should be less than oral intake of this herb. Even though all other herbal formulations showed no acute toxicity and mutagenic effect, the chronic toxicity studies are needed especially when the products are expected to be consumed for a long period.

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P 14 Safety Evaluation of Ran-jued (*Thunbergia laurifolia*) Tea

Sarunya Laovitthayanggoon[1], Onanong Charoenkul[2], Porntip Supavilai[1]and Auratai Aramphongphan [1,2]

[1]*Department of Pharmacology, Faculty of Science, Mahidol University*
[2]*Toxicology graduate program, Faculty of Science, Mahidol University*
E-mail: scaap@mahidol.ac.th

Abstract

Introduction: In Thailand, most of scientific researches on herbs and herbal products are concentrated mainly on their efficacy, identification of active compounds and purification of active compounds. A few studies aim for safety of herbal products.

Objective: This study attempted to evaluate the safety of common Thai herbal teas by studying their genotoxic potential and quantitative the amount of mycotoxins and heavy metals present in each tea product.

Materials and Methods: Five commercial brands of Ran-jued tea were randomly selected for safety evaluation. The levels of aflatoxin B_1 (AFB_1) contamination and heavy metal residues including cadmium (Cd), mercury (Hg) and lead (Pb) were determined. Genotoxicity testing was conducted to assess the mutagenic potential of Ran-jued tea. The bacterial reverse mutation test (Ames' test) using *Salmonella typhimurium* TA98 and TA100 with and without an extract of rat liver homogenate (S9 fraction) was used to detect the mutagenicity of Ran-jued tea extracts. The antioxidant activity of Ran-jued tea extracts was determined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay.

Results: The results showed that AFB_1 contaminated levels were above the current legislative level permissible of Thailand (20 ng/g). The Hg and Pb residual were in the acceptable levels (i.e., Hg levels <10 and Pb<10 mg/kg Ran-jued) while some of trademark contained high level of Cd that was above the acceptable standard (<0.3 mg/kg). The results indicated no mutagenic effect in the Ran-jued tea-extract tested. Finally, the DPPH assay showed the antioxidant activity (EC_{50}), ranging from 0.05-0.56 mg/ml Ran-jued tea extracts.

Conclusion: AFB_1 and cadmium contaminations in commercial Ran-jued tea were above the permissible level whereas those of mercucery and lead were acceptable. And the results of Ames'test exhibited no mutagenic effect of all Ran-jued Tea extracts.

Keywords: Ran-jued tea (*Thunbergia laurifolia*), Ames's test, antioxidant, Aflatoxin B_1, and heavy metals

Introduction

Herbal products have received a great deal of attention worldwide, for their preventive effects on major chronic diseases such as cancer and Parkinson's diseases.

The Thai consumption of herbal products has been on the increasing trend as shown by value of Thai herbal products more than 48,000 million bahts (ศูนย์วิจัยกสิกรไทย, 2548). The Thai government announces the policy for promoting Thai herbal preparations for local consumption and for export. In the strategic development, the high quality products having scientifically proven support for health claims are needed to compete with the global market and make Thai herbal preparations well accepted by other foreign countries.

Herbal preparations became popular because the use is simple and inexpensive. Herbal products uses are primarily based on tradition or scientific theories. They often have not been thoroughly tested for their safety and effecacy in human and most of the consumers are convinced that natural products are non-toxic because of their natural origin. However, many factors were involved in herbal adverse effects, i.e. overdosing, prolonged use, misuse, residue and contaminants.

The aim of this study was to evaluate the safety of Ran-jued (*Thunbergia laurifolia)* tea by measuring the carcinogenic mycotoxin, aflatoxin B_1 (AFB_1) contamination, heavy metals residuals such as carcinogenic metal, cadmium, toxic mercury and lead and the evaluation of genotoxicity potential. The antioxidant activity, which is involved in pharmacologic property of Ran-jued tea was also determined. The information obtained from the study will provide useful information for safety profile of Ran-jued tea and ensure the safety of this herbal preparation for public.

Materials and methods

Five commercial brands of Ran-jued (*Thunbergia laurifolia*) were randomly selected for investigation.

Preparation of sample for mutagenic assay and antioxidant assay:

Hot water was prepared by boiling water to 100°C and let stand for 3 minutes to bring the temperature down to 80°C, the optimum temperature for tea infusion. The hot water, 120 ml, was poured into a 200 ml cup, simmered the tea pouch (120 ml/pouch) for 3 minutes. Then tea bag was gently squeezed to let the active ingredients fully release into water. After cooling to room temperature and filtering, the supernatant was concentrated by a rotary evaporator under reduced pressure at 50°C and dried by lyophilizer.

Heavy metal determination

Ran-jued tea (0.5 g. dry weight) was digested in a 50 ml PE Teflon pressurized vessels with 9 ml of 65% HNO_3 by a digestive microwave (CEM Model, MarsX,). After digestion, 1 ml of H_2O_2 was added to the digested sample and then filtered through a Whatman No. 1 filter paper. Finally the volume was made up to 25 ml with milli-Q water. The levels of heavy metals, Cd, Hg and Pb were determined by Atomic Absorption Spectrophotometer (AAs).

Aflatoxin determination:

Sample preparation: AFB_1 was extracted by putting 20 gm of grounded Ran-jued tea into an Erlenyer flask. Then 100 ml of 70% MeOH was added and the flask was rovolved at 300 rpm for 30 min and then mixture filtered. The AFB_1 level was detected by DOA-Aflatoxin ELISA Test Kit (กรมวิชาการเกษตร, 2547)

ELISA protocol: Added 50 µl of AFB_1 standards into the antibody coated wells and 50 ul of diluted sample into the other wells followed by adding 50 ul of AFB1-HRP conjugate to each well, slightly shake then incubated at room temperature for 30 min. Dumped the contents of the well into the appropriate waste container and washed the plate 3- 5 times by 0.01M phosphate buffer saline + 0.5% Tween 20 (PBS-T). Added 100 ul of substrate (Tetramethylbenzidine) (KPL Inc.) to the well, incubated 10 min at room temperature then added 100 µl of stopping solution (0.3M Phosphoric acid). Read the color at 450 nm using the automated microplate reader.

Antioxidant activity

DPPH radical scavenging assay radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The change

in color (from deep violet to light yellow) was measured at 515 nm on an automated microplate reader. Radical scavenging activity of extracts was measured by a method of Brand-Williams, Cuvelier, and Berset (1995) with slight modification.

The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50 % (EC_{50}) value. Each sample was determined for EC_{50} graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration

Mutagenicity testing by Ames test: The bacterial reverse mutation test (Ames' test) was used to detect genotoxic properties of the water extracts of Rang-jued. The concentrations of Rang-jued were 0.125, 0.25, 0.5, 1.0 and 2.0 mg/plate. The 0.1 ml. of crude extract of herb tests, 0.1 ml of tester strain, and 0.5 ml of buffer or S9 fraction were mixed and incubated at 37^0 C, 20 minutes before pouring into minimal glucose agar plate for incubation at 37^0 C, 48 hr. Finally, the revertant colonies were counted to compare with negative and positive controls.

Results and Discussion

The results revealed that commercial brands of Ran-jued were contaminated with the detectable amount of AFB_1 ranging from 24.04-59.51 ppb, which were above the current legislative level permissible of Thailand (20 ppb). The heavy metals contamination were in the ranges of 0.5-1.1 for Cd, < 0.10 for Hg, and <0.02 mg/kg for Pb. Some of commercial brands contained high level of Cd that was above the acceptable standard (<0.3 mg/kg) while the detected levels of Hg and Pb were in the acceptable levels. No mutagenicity was found in Rang-jued tea extracts at the concentrations range from 0-2 mg/plate.

Conclusion

Aflatoxin contamination is a problem of various herbal drugs. In this study AFB_1 content up to 59.51 ppb, the content higher than permissible level in Thailand was detected in all of the samples tested. Therefore the process of cultivation, harvesting and drying should be improved and the application of Good Agricultural Practice principles may help to reduce mycotoxin contamination of herbal products. Results of heavy metals analysis demonstrated high amount of Cd in all selected Rang-jeud tea. This Cd may taken by plants during cultivation and accumulate in their leaves. Cd is human carcinogen and is therefore of concern in long-term herb consumers.

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P15 No Direct Hepatotoxic Potential Following a Multiple-Low Dose Paraquat Exposure in Rat as Related to Its Bioaccumulation

Varaporn Podprasart [a], Jutamaad Satayavivad [a,b], Suda Riengrojpitak [c], Prapin Wilairat [d], Winai Wananukul [e], Pranee Chavalittumrong [f], Songpol Chivapat [f], Krongtong Yoovathaworn [a,b]

[a] Graduate Program in Toxicology, [b] Departments of Pharmacology, [c] Pathobiology, [d] Chemistry, Faculty of Science, Mahidol University [e] Division of Pharmacology and Toxicology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, [f] Department of Medical Science, Ministry of Public Health
E-mail: podprasart6@yahoo.com

Abstract

Introduction: Paraquat (PQ) is a well-known pulmonary toxicant bipyridyl herbicide commonly used in agricultural countries. PQ is also classified as a *"direct hepatotoxicant"*. Occupational exposure is a multi-low dose exposure.

Objective: To examine the effect of multiple low doses of PQ on the liver function and xenobiotic-metabolizing enzyme activities, and to correlate the effects with its tissue accumulation in male Wistar rats.

Materials and methods: PQ, dose range 4.0 - 6.0 mg/kg/d, was injected subcutaneously. The liver function, xenobiotic-metabolizing enzyme activity, lung and liver morphology and accumulation of PQ in tissues were determined.

Results: PQ-treatment caused a dose- and time-dependent reduction of ALT, AST, hypobilirubinemia and hypoalbuminemia with no alteration in the liver morphology. The activity of CYP1A1-related 7-ethoxyresorufin-O-deethylase was reduced following the highest dose of PQ. Plasma and tissue concentrations of PQ analyzed by HPLC were dose- and time-dependent showing 13 times higher in the lung than that in the liver whereas it was undetectable in the plasma at the same time point.

Conclusion: Multi-low doses PQ affect certain synthetic function of the liver or activity of some hepatic xenobiotic-metabolizing enzymes. Minimal PQ accumulation in the liver is one of the explanations for the lack of cytotoxic hepatic injury. Plasma PQ concentration may not be a good marker of exposure and toxicity after a prolonged exposure.

Keywords: Paraquat; Hepatotoxicity; Enzymes; Accumulation; CYP

1. Introduction

Paraquat (PQ; 1,1'-dimethyl-4,4'-bipyridinium ion, methyl viologen), a non-selective contact herbicide, causes toxicity mainly to the lung, and usually multisystem failure. Occupational exposure by workers in the agricultural areas probably occurs at low doses of multiple exposures. PQ is an agent inducing cholestasis. The study on the toxic effect of PQ after long-term exposure in humans is lacking. The present study was, therefore, conducted to investigate the multi-dose effect of PQ on the liver function and xenobiotic-metabolizing enzyme activities, and to correlate the effects with its tissue accumulation. A multi-low dose PQ exposure model used herein might resemble occupational exposure.

Materials and methods

Male Wistar rats (120-140 g) were purchased from the National Laboratory Animal Centre, Thailand and allowed acclimatization for 1 week. All animals were cared for and treated in accordance with NIH and Mahidol University Animal Care and Use Committee guidelines. PQ was injected sc at the doses of 4.0, 5.0, and 6.0

mg/kg/d for 7 days. Plasma Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), total bilirubin, BUN, creatinine, total protein and albumin concentrations were determined. Liver was morphologically evaluated using standard H&E staining. Liver samples were studied for CYP activities including *O*-deethylation of ethoxyresorufin (CYP1A1), *p*-nitrophenol hydroxylation, (CYP2E1) and erythromycin *N*-demethylase activity (CYP3A4) and microsomal protein content. The plasma and tissue PQ were determined by the method described by Fuke et al (2002) and Corasaniti et al (1990). Plasma and tissue PQ concentrations after single and multi-dose exposures (7 days) of 6 mg/kg PQ, sc., were determined at pre-assigned time points post dose.

Statistical comparisons of the results were carried out by ANOVA and post hoc analysis. *P* values less than 0.05 were considered to be significant.

Results

Decreased plasma ALT and AP and a dose-dependent decrease in the level of both albumin and total bilirubin were observed (Table 1). A significant decrease in BUN and serum creatinine levels, but not the total plasma protein level, was seen at the PQ dose of 6.0 mg/kg/d.

PQ treatment for 7 days resulted in extensive pulmonary hemorrhage, thickening of alveolar septum and infiltration of inflammatory cells (not shown). The hepatocytes of the PQ-treated groups were still intact. Significant decrease was found only in the ethoxyresorufin-*O*-deethylase activity in PQ treated groups (Table 2). PQ caused reduction the hepatic microsomal protein content.

Accumulation of PQ in tissues (Figure 1) was dose-related. High PQ concentrations were detected in the lung compared with that of the liver. No difference in the plasma concentration-time profiles *of* PQ after single dose and multi-dose exposures at any time points.(Fig.2). Lung and liver PQ concentrations after multi-low dose PQ exposure were much higher than those obtained from a single dose at all times.

Discussion

The results of chronic PQ toxicity study by Suriyo (2001) gave support to the use of rats as an animal model for PQ-induced hepatotoxic effect. Multi-low dose PQ did not seem to be a hepatotoxic agent. No increase in the activity of the marker enzymes for liver damage but a decrease in either plasma albumin or total bilirubin levels was found. At low dose, PQ did not affect either CYP2E1 or CYP3A4 activity but it caused a dose-dependent reduction in the CYP1A1 activity. Such effect might have both clinical and toxicological significances in terms of therapeutic outcome and risk of toxic exposure. The mechanism by which PQ reduces CYP1A1 activity is unclear and needs to be explored.

Nearly undetectable PQ at 24 hours postdose demonstrated that plasma PQ concentration, which has been thought to be a good marker for PQ exposure and toxicity after a single high dose in man, may not be as useful after low multi-dose exposure. Multi-low dose exposure could increase lung PQ concentration to the toxic range. Low hepatic PQ concentration may be due to the absence of specific transporters or rapid disappearance of PQ from the liver via biliary excretion and biotransformation to a less toxic compound. PQ-induced lipid peroxidation might be suppressed by the antioxidant enzyme systems that were plentiful in the liver.

Conclusion:

The study on the multi-low dose PQ toxicity indicated that this herbicide might affect certain synthetic function of the liver or activity of some hepatic xenobiotic-metabolizing enzymes. Resistance to the toxic effect of PQ by the liver was probably due to low bioaccumulation of PQ in this organ. In addition, plasma PQ concentration does not indicate the degree of exposure and severity of PQ toxicity.

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Table 1 The effects of PQ at various doses on blood chemistry

Parameters	PQ (mg/kg/d)			
	0 (10)	4.0 (10)	5.0 (9)	6.0 (9)
AST (U/L)	77.10 ± 4.47	71.00 ± 3.96	71.22 ± 4.64	55.33 ± 3.28**
ALT (U/L)	26.30 ± 1.33	24.20 ± 0.89	25.56 ± 1.22	23.78 ± 2.13
AP (U/L)	156.0 ± 5.47	161.0 ± 6.63	157.6 ± 5.57	144.3 ± 8.16
Total bilirubin (mg/dl)	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01*
Albumin (mg/dl)	4.10 ± 0.11	3.85 ± 0.11	3.72 ± 0.14*	3.33 ± 0.12***
Total protein (g/dl)	6.34 ± 0.19	6.41 ± 0.19	6.54 ± 0.18	6.72 ± 0.13
BUN (mg/dl)	19.00 ± 1.05	17.96 ± 0.51	17.87 ± 0.22	16.51 ± 0.45*
Creatinine (mg/dl)	0.52 ± 0.01	0.52 ± 0.01	0.50 ± 0.01	0.43 ± 0.01***

PQ 4.0, 5.0, and 6.0 mg/kg/d, once daily, for 7 days. Values are mean ± S.E.M.

* and ***, represent *p*-values of less than 0.05 and 0.001, respectively

Table 2 Effect of multi-low dose PQ on hepatic CYP1A1, CYP2E1, and CYP3A4

Parameter	PQ (mg/kg/d)			
	0	4.0	5.0	6.0
EROD	0.11 ± 0.01	0.10 ± 0.01	0.05 ± 0.01***	0.03 ± 0.00***
PNPH	5.07 ± 0.21	4.72 ± 0.22	5.09 ± 0.46	5.87 ± 0.40
ERY	1.09 ± 0.06	1.29 ± 0.07	1.03 ± 0.04	1.11 ± 0.07
Microsomal protein	19.13 ± 0.65	16.29 ± 0.83*	17.42 ± 0.60	14.73 ± 0.73***

PQ once daily, for 7 days. Values are mean ± S.E.M. from 8 animals.

* and ***, represent *p*-values of less than 0.05 and 0.005, respectively

Ethoxyresorufin-*O*-deethylation (EROD) nmole of resorufin formed/mg protein/min

p-Nitrophenol hydroxylation (PNPH) = nmole of 4-nitrocatechol formed/mg protein/min

Erythromycin-*N*-demethylase (ERY) = nmole of formaldehyde formed/mg protein/min

microsomal protein content = mg/g wet liver weight

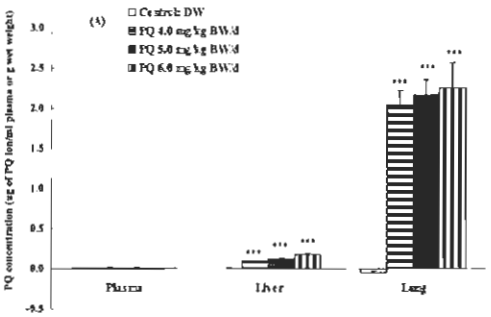

Fig. 2

Bioaccumulation of PQ in the plasma and tissues after multi-low dose administration (4.0, 5.0, and 6.0 mg/kg/d, sc) for 7 days. Bar graphs represent mean ± S.E.M, n=8 each. The asterisks, ***, represent *p*-value less than 0.005.

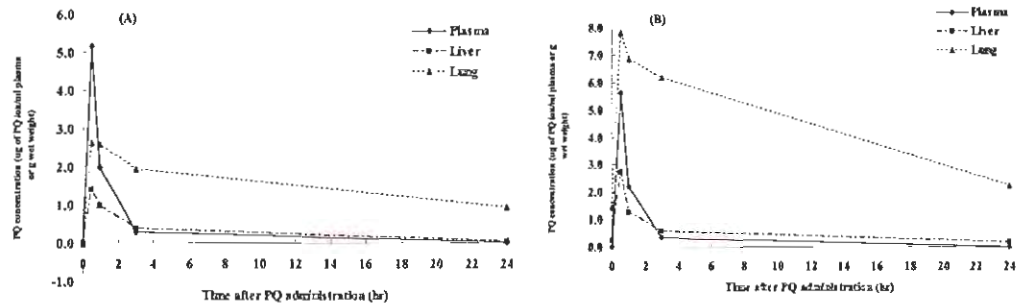

Fig. 2 Concentration-time profiles of PQ in the plasma, liver, and lung after a single dose, 6.0 mg/kg (A), and multi-dose, 6.0 mg/kg/d (B), sc, 7 consecutive days.

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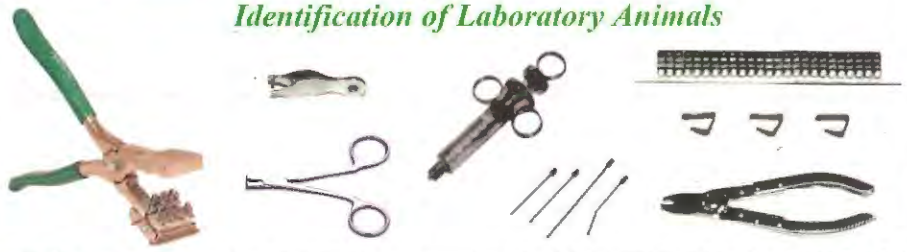

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