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งานประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย

ครั้งที่ 42

Precision Medicine

From Research *to* Clinical Implementations

19–21 พฤษภาคม 2564

ณ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

PROCEEDINGS

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Precision Medicine from Research to Clinical Implementations

จัดการประชุมโดย

ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

ร่วมกับ

สมาคมเภสัชวิทยาแห่งประเทศไทย

บรรณาธิการ

ศาสตราจารย์ ดร. ภก. วีรพล คู่คงวิริยพันธุ์

ดร. ภญ. นนทญา นาคคำ

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คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

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สารบัญ

สารจากนายกสมาคมเภสัชวิทยาแห่งประเทศไทย

เรียน สมาชิกสมาคมเภสัชวิทยาแห่งประเทศไทย และผู้เข้าร่วมประชุมทุกท่าน

สมาคมเภสัชวิทยาแห่งประเทศไทยขอต้อนรับทุกท่านเข้าสู่การประชุมในรูปแบบของ New normal สมาคมฯ จัดการประชุมวิชาการประจำปีมาอย่างต่อเนื่อง ครั้งนี้เป็นการประชุมครั้งที่ 42 ซึ่งเป็นการประชุมที่ถูกเลื่อนมาจากปีที่แล้วสืบเนื่องจากสถานการณ์การแพร่ระบาดของโรค Coronavirus Disease (COVID-19) นับจนถึงบัดนี้ ประเทศไทยได้เกิดการระบาดของโรค COVID-19 เป็นระลอกที่สอง แต่ในครั้งนี้ ด้วยความมุ่งมั่นของภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น เจ้าภาพจัดงานประชุม จึงทำให้การประชุมในครั้งนี้สามารถเกิดขึ้นได้โดยเป็นการจัดในรูปแบบออนไลน์ตลอดการประชุม

สำหรับการประชุมในครั้งนี้ได้รับเกียรติจาก ศาตราจารย์ นายแพทย์วรศักดิ์ โชติเลอศักดิ์ ภาควิชากุมารเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย บรรยายปาฐกถาเกียรติยศ รองศาตราจารย์ ดร.จิรวัฒก์ สดาวงศ์วิวัฒน์ (Dr. Chiravat Sadavongvivad Memorial Lecture) และการบรรยายพิเศษจาก รองศาสตราจารย์ ดร. เภสัชกรหญิงจินตนา สัตยาศัย จากภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น เนื่องในโอกาสได้รับรางวัลเกียรติยศ ศาสตราจารย์ นายแพทย์อวย เกตุสิงห์ ประเภทนักเภสัชวิทยาดีเด่น อีกทั้งยังมีการบรรยายโดย Associate Professor Hiroto Hatakeyama จาก Graduate School of Pharmaceutical Sciences, Chiba University, ประเทศญี่ปุ่น ภายใต้โครงการความร่วมมือระหว่างสมาคมเภสัชวิทยาแห่งประเทศไทยกับ The Japanese Society for the study of Xenobiotics (JSSX) รวมถึงนักวิจัยที่มีผลงานโดดเด่นอีกจำนวนมาก สมาคมฯ หวังว่าทุกท่านจะเพลิดเพลินและได้รับประโยชน์จากการฟังบรรยายในครั้งนี้

ในนามของสมาคมฯ ขอขอบพระคุณ ศาสตราจารย์ ดร. เภสัชกรหญิงวิจิตรา ทัศนียกุล ที่รับเป็นประธานจัดงานประชุมในครั้งนี้ ขอขอบพระคุณวิทยากรทุกท่าน คณะกรรมการจัดงานประชุม ผู้เข้าร่วมประชุม และหน่วยงานต่าง ๆจากทั้งภาครัฐและภาคเอกชนที่ให้การสนับสนุนการจัดประชุมวิชาการในครั้งนี้

รองศาสตราจารย์ ดร. เภสัชกรหญิงรัตติมา จีนาพงษา
นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

สารจากประธานการจัดงานประชุมวิชาการประจำปี

เรียน นายกสมาคมเภสัชวิทยาแห่งประเทศไทย ท่านอาจารย์อาวุโส วิทยากร ผู้เข้าร่วมประชุม และสมาชิกสมาคมเภสัชวิทยาฯ ทุกท่าน

ในนามประธานคณะกรรมการจัดการประชุมฯ ดิฉันมีความยินดีต้อนรับทุกท่านที่เข้าร่วมประชุมวิชาการประชุมครั้งนี้ การประชุมวิชาการครั้งนี้เป็นการประชุมครั้งที่ 42 ซึ่งเป็นการประชุมที่ถูกเลื่อนมาจากปีที่แล้วสืบเนื่องจากสถานการณ์การแพร่ระบาดของโรค Coronavirus Disease (COVID-19) ครั้งแรกในประเทศไทยเมื่อปี 2563 ซึ่งเดิมทีคณะกรรมการจัดการประชุมมีความตั้งใจที่จะจัดการประชุม onsite ที่อุทยานวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น เพื่อให้สมาชิกฯ และผู้เข้าร่วมประชุมทุกท่านได้มีโอกาสพบปะ พูดคุยและแลกเปลี่ยนความเห็นด้านวิชาการ แต่เนื่องจากเกิดสถานการณ์แพร่ระบาด COVID-19 รอบ 2 อีกครั้งเมื่อต้นปีนี้ทำให้คณะกรรมการจัดการประชุมและกรรมการสมาคมฯ มีมติร่วมกันที่จะจัดการประชุมในรูปแบบ online ผ่านระบบ zoom application ซึ่งหัวข้อใหญ่ของการประชุมครั้งนี้คือ “Precision Medicine from Research to Clinical Implementations” โดยมีหัวข้อย่อยของการประชุมหลากหลายครอบคลุมความรู้ precision medicine หลากหลาย ตั้งแต่การวิจัยในห้องปฏิบัติการ ไปจนถึงการนำไปใช้ประโยชน์ทางคลินิก และการกำหนดนโยบายสาธารณสุขของประเทศ ทั้งนี้การประชุมในครั้งนี้ ได้รับเกียรติจากวิทยากรที่มีชื่อเสียงหลายท่าน อาทิเช่น ศ.นพ.วรศักดิ์ โชติเลอศักดิ์ ภาควิชากุมารเวชศาสตร์จุฬาลงกรณ์มหาวิทยาลัย องก์ปาฐกถา ของปาฐกถาเกียรติยศ (Dr. Chiravat Sadavongvivad Memorial Lecture) รวมทั้งวิทยากรรับเชิญจากต่างประเทศ Associate Professor Hiroto Hatakeyama จาก Graduate School of Pharmaceutical Sciences, Chiba University, ประเทศญี่ปุ่น ภายใต้โครงการความร่วมมือระหว่างสมาคมเภสัชวิทยาแห่งประเทศไทยกับ The Japanese Society for the study of Xenobiotics (JSSX) และ Dr. Ong Choon Kiat , National Cancer Centre, Singapore

การประชุมครั้งนี้ได้รับการตอบรับจากสมาชิก นิสิต นักศึกษา ตลอดจนผู้สนใจเป็นอย่างดี มีผู้ลงทะเบียนเข้าร่วมประชุมกว่า 200 ท่าน คณะกรรมการจัดการประชุมได้จัดให้มี poster session เพื่อให้นักเภสัชวิทยาทุกท่านได้มีโอการนำเสนอผลงายวิจัย และแลกเปลี่ยนความรู้ซึ่งกันและกัน โดย poster session นี้มีรูปแบบการนำเสนอแบบ new normal ซึ่งคณะกรรมการจัดการประชุมฯ หวังว่าทุกท่านจะได้รับประโยชน์จากการเข้าร่วมการประชุมครั้งนี้อย่างเต็มที่

ท้ายนี้ดิฉันขอขอบพระคุณวิทยากรทุกท่าน คณะกรรมการบริหารสมาคมเภสัชวิทยา ฯ คณะกรรมการจัดการประชุม ท่านที่ปรึกษาสมาคมเภสัชวิทยา ฯ รวมทั้งบุคคลากร และเจ้าหน้าที่ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ รวมทั้งภาคเอกชนและหน่วยงานต่าง ๆ ที่มีส่วนร่วมให้การจัดการประชุมครั้งนี้สำเร็จลุล่วงไปด้วยดี

ด้วยความปรารถนาดี

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ประธานคณะกรรมการจัดการประชุมวิชาการ

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งานประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 42

จัดโดย

ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

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รองศาสตราจารย์สุดา วรรณประสาท	กรรมการ
รองศาสตราจารย์ลัดดาวัลย์ เส็งกันไพร	กรรมการ
รองศาสตราจารย์เอื้อมเดือน ประวาฬ	กรรมการ
ผู้ช่วยศาสตราจารย์ปณต ตั้งสุจริต	กรรมการ
ผู้ช่วยศาสตราจารย์ศิริมาศ กาญจนวาศ	กรรมการ
ผู้ช่วยศาสตราจารย์ศริญญา คงเพชร	กรรมการและเลขานุการ
อาจารย์นนทญา นาคคำ	กรรมการและผู้ช่วยเลขานุการ

คณะแพทยศาสตร์ มหาวิทยาลัยสยาม

ศาสตราจารย์เกียรติคุณ บุญเจือ ธรณินทร์	กรรมการ

วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต

ศาสตราจารย์เกศรา ณ บางช้าง	กรรมการ
รองศาสตราจารย์วรรณา ชัยเจริญกุล	กรรมการ

คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

รองศาสตราจารย์สุพีชา วิทยเลิศปัญญา	กรรมการ
ผู้ช่วยศาสตราจารย์วัชรี ลิมปนสิทธิกุล	กรรมการ

สถาบันการแพทย์จักรีนฤบดินทร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

รองศาสตราจารย์ณัฐวุธ สิบหมู่	กรรมการ
รองศาสตราจารย์พรพรรณ วิวิธนาภรณ์	กรรมการ

คณะแพทยศาสตร์ ศิริราชพยาบาล มหาวิทยาลัยมหิดล

รองศาสตราจารย์ศิวนนท์ จิรวัฒโนทัย กรรมการ

คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

รองศาสตราจารย์ชลภัทร สุขเกษม กรรมการ

คณะเภสัชศาสตร์ มหาวิทยาลัยบูรพา

รองศาสตราจารย์มยุรี ตันติสิระ กรรมการ

คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร

รองศาสตราจารย์รัตติมา จีนาพงษา กรรมการ

คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

รองศาสตราจารย์ศุภนิมิต ทีฆชุณหเถียร กรรมการ

คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ผู้ช่วยศาสตราจารย์วันดี อุดมอักษร กรรมการ

คณะกรรมการพิจารณาผลงานการประกวด

คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

ศาสตราจารย์วีรพล คู่คงวิริยพันธุ์	ประธานกรรมการ
ศาสตราจารย์วิจิตรา ทัศนียกุล	กรรมการ
รองศาสตราจารย์ลัดดาวัลย์ เส็งกันไพร	กรรมการ
ผู้ช่วยศาสตราจารย์ศริญญา คงเพชร	กรรมการและเลขานุการ
อาจารย์นนทญา นาคคำ	กรรมการและผู้ช่วยเลขานุการ

คณะแพทยศาสตร์ มหาวิทยาลัยสยาม

ศาสตราจารย์เกียรติคุณบุญเจือ ธรณินทร์	กรรมการ

วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

รองศาสตราจารย์ พลตรี บพิตร กลางกัลยา	กรรมการ

วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์ ศูนย์รังสิต

ศาสตราจารย์เกศรา ณ บางช้าง	กรรมการ
รองศาสตราจารย์วรรณา ชัยเจริญกุล	กรรมการ

คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

รองศาสตราจารย์สุพีชา วิทยเลิศปัญญา	กรรมการ
รองศาสตราจารย์สุมนา ชมพูทวีป	กรรมการ
ผู้ช่วยศาสตราจารย์วัชรี ลิมปนสิทธิกุล	กรรมการ

คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

รองศาสตราจารย์สุวรา วัฒนพิทยกุล	กรรมการ

คณะแพทยศาสตร์ ศิริราชพยาบาล มหาวิทยาลัยมหิดล

รองศาสตราจารย์ศิวนนท์ จิรวัฒโนทัย	กรรมการ

สถาบันการแพทย์จักรีนฤบดินทร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

รองศาสตราจารย์ณัฐวุธ สิบหมู่ กรรมการ
รองศาสตราจารย์พรพรรณ วิวิธนาภรณ์ กรรมการ

คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

รองศาสตราจารย์ชลภัทร สุขเกษม กรรมการ

คณะเภสัชศาสตร์ มหาวิทยาลัยบูรพา

รองศาสตราจารย์มยุรี ตันติสิระ กรรมการ

คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร

รองศาสตราจารย์รัตติมา จีนาพงษา กรรมการ
รองศาสตราจารย์นันทีทิพ ลิ้มเพียรชอบ กรรมการ
ผู้ช่วยศาสตราจารย์สกลวรรณ ประพฤติบัติ กรรมการ

คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

รองศาสตราจารย์ศุภนิมิต ทีฆชุณหเถียร กรรมการ

คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

ผู้ช่วยศาสตราจารย์วันดี อุดมอักษร กรรมการ

กำหนดการงานประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 42 (ออนไลน์)

"Precision Medicine from Research to Clinical Implementations" (online conference)

ระหว่างวันที่ 19-21 พฤษภาคม พ.ศ. 2564

คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

วันพุธที่ 19 พฤษภาคม 2564

08.00-08.30	ลงทะเบียน
08.30-09.30	พิธีเปิด ● กล่าวรายงานโดย ประธานคณะกรรมการจัดการประชุม ศ.ดร.วิจิตรา ทัศนียกุล ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ● กล่าวเปิดงาน โดย นายกสมาคมเภสัชวิทยาแห่งประเทศไทย รศ.ดร.รัตติมา จีนาพงษา
09.30-10.30	**ปาฐกถาเกียรติยศ (Dr. Chiravat Sadavongvivad Memorial Lecture)** **"Integrating Genomics Medicine into Healthcare"** ศ.นพ.วรศักดิ์ โชติเลอศักดิ์ ภาควิชากุมารเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
10.30-10.45	พักรับประทานอาหารว่าง/ชมวิดิทัศน์ ข้อมูลผลิตภัณฑ์ เครื่องมือวิจัยทางด้านเภสัชวิทยาและการแพทย์แม่นยำ
10.45-11.45	**Plenary Lecture 1** **"Evaluation of Pharmacokinetics and Efficacy of Immune Checkpoint Inhibitors ~ Lessons from Animal Tumor Models"** Assoc. Prof. Hiroto Hatakeyama Graduate School of Pharmaceutical Sciences, Chiba University, Japan (Under collaboration between The Pharmacological and Therapeutic Society of Thailand and The Japanese Society for the study of Xenobiotics (JSSX))
11.45-12.15	**พิธีมอบรางวัลและนำเสนอผลงาน "รางวัลนักเภสัชวิทยาดีเด่น ประจำปี 2563" จากสมาคมเภสัชวิทยาแห่งประเทศไทย**
12.15-13.00	**Lunch Symposium 1 (GenePlus)** **Precision Medicine Initiative: From Science to Clinical Outcome** *วิทยากร:* Kevin Lin, Microarray Product Manager/South East Asia, Thermo Fisher Scientific

Symposium 1: Pharmacogenomics from Research to Clinical and Healthcare Policy Implementation	
(13.00-14.00)	ผู้ดำเนินการอภิปราย: ดร.นนทญา นาคคำ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
13.00-13.20	• **Pharmacogenomics of severe cutaneous adverse drug reactions and their implementation in healthcare system policy** *วิทยากร:* ศ.ดร.วิจิตรา ทัศนียกุล ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
13.20-13.40	• **Pharmacogenomics Thailand: Research and clinical implementations** *วิทยากร:* รศ.ดร.ชลภัทร สุขเกษม ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล
13.40-14.00	• **Clinical pharmacogenomics: Application in transplantation** *วิทยากร:* ผศ.พญ.ปาจรีย์ จริยวิลาศกุล ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
14.00-15.15	**Poster Presentation and Discussion (online presentation)**
15.15-15.30	พักรับประทานอาหารว่าง/ชมวิดิทัศน์ ข้อมูลผลิตภัณฑ์ เครื่องมือวิจัยทางด้านเภสัชวิทยาและการแพทย์แม่นยำ
Symposium 2: Precision Medicine in Cancer Therapy	
(15.30-16.30)	ผู้ดำเนินการอภิปราย: ผศ.ดร.ศริญญา คงเพชร ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
15.30-15.50	• **Precision medicine and innovative treatment for cancer (Dependency of cholangiocarcinoma on cyclin D-dependent kinase activity)** *วิทยากร:* รศ.ดร.ศิวนนท์ จิรวัฒโนทัย ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
15.50-16.10	• **Targetable fusion genes in cancers: the NGS approach for precision diagnosis and treatment** *วิทยากร:* ผศ.ดร.ศริญญา คงเพชร ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
16.10-16.30	• **Precision oncology in clinical practice** *วิทยากร:* ผศ.พญ.เอื้อมแข สุขประเสริฐ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
16.30-17.30	ประชุมใหญ่สามัญประจำปี 2564 สมาคมเภสัชวิทยาแห่งประเทศไทย (เรียกประชุมครั้งที่ 1)

วันพฤหัสบดีที่ 20 พฤษภาคม 2564

08.30-09.00	**ลงทะเบียน**
09.00-10.00	**Plenary Lecture 2** **"Translating Discoveries to Clinical Applications in Treating Asian Lymphoma – From Bench to Bedside and Back "** *Dr. Ong Choon Kiat, National Cancer Centre, Singapore*
10.00-10.30	พักรับประทานอาหารว่าง/ ชมวิดิทัศน์ ข้อมูลผลิตภัณฑ์ เครื่องมือวิจัยทางด้านเภสัชวิทยาและการแพทย์แม่นยำ
Symposium 3: Nutraceuticals and Personalized Lifestyle Medicine (10.30-12.00) ผู้ดำเนินการอภิปราย: รศ.ดร.จินตนา สัตยาศัย ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น	
10.30-11.00	• **Doctor's concept of patient care and the principles of personalized nutrition and/or personalized nutraceuticals** *วิทยากร:* นพ.ก้องเกียรติ เกษเพ็ชร์ รพ.กรุงเทพพัทยา ชลบุรี
11.00-11.30	• **Nutraceuticals targeting gut microbiota for precision medicine** *วิทยากร:* ผศ.ดร.สยาม ภพลือชัย สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
11.30-12.00	• **Rice as an example of a personalized lifestyle medicine for NCDs** *วิทยากร:* รศ.ดร.จินตนา สัตยาศัย ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
12.00-13.30	**Lunch Symposium 2 (Greater Pharma)** **Mite Vaccine: From Research to Clinical Practice** *วิทยากร:* ศ.เกียรติคุณ นพ.บุญเจือ ธรณินทร์ คณะแพทยศาสตร์ มหาวิทยาลัยสยาม รศ.ดร.นพ.พงศกร ตันติลิปิกร ภาควิชา โสต นาสิก ลาริงซ์วิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

Symposium 4: COVID-19 Current Status and Future Perspectives	
(13.30-14.30)	ผู้ดำเนินการอภิปราย: ดร.นนทญา นาคคำ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
13.30-13.50	• **Virology and immunology in SARS-CoV2** *วิทยากร:* ผศ.ดร.ป๋วย อุ่นใจ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
13.50-14.10	• **Pharmacological treatment of COVID-19** *วิทยากร:* ดร.นนทญา นาคคำ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
14.10-14.30	• **Current status of COVID-19 vaccine development** *วิทยากร:* ดร.สมชัยยา สุริฉันท์ สถาบันวิจัยและพัฒนาองค์การเภสัชกรรม
14.30-15.00	พักรับประทานอาหารว่าง/ชมวิดิทัศน์ ข้อมูลผลิตภัณฑ์ เครื่องมือวิจัยทางด้านเภสัชวิทยาและการแพทย์แม่นยำ
Symposium 5: Redox Signaling as Therapeutic Target for Oxidant Ridden Diseases	
(15.00-16.00)	ผู้ดำเนินการอภิปราย: รศ.ดร.เอื้อมเดือน ประวาฬ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
15.00-15.20	• **Modulation of metabolic cellular sensor as a strategy in tumor suppression** *วิทยากร:* รศ.ดร.เอื้อมเดือน ประวาฬ ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
15.20-15.40	• **The role of antioxidant defense in a precision approach against skin aging** *วิทยากร:* รศ.ดร.พญ.อุไรวรรณ พานิช ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ ศิริราชพยาบาล มหาวิทยาลัยมหิดล
15.40-16.00	• **Ferroptosis and development of lipid radical inhibitors for diseases with lipid oxidation** *วิทยากร:* รศ.ดร.นพวรรณ ภู่มาลา มอลาเลส ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
16.00-16.30	**พิธีมอบรางวัลการนำเสนอผลงานวิจัย และพิธีปิดการประชุม**
16.30-17.30	**ประชุมใหญ่สามัญประจำปี 2564 สมาคมเภสัชวิทยาแห่งประเทศไทย** (เรียกประชุมครั้งที่ 2 ในกรณีที่การประชุมครั้งที่ 1 สมาชิกฯ เข้าร่วม ไม่ครบองค์ประชุม)

วันศุกร์ที่ 21 พฤษภาคม 2564

08.30-09.00	**ลงทะเบียน**
09.00-12.00	**อภิปราย แลกเปลี่ยนประสบการณ์ การเรียนการสอนและงานวิจัยด้านเภสัชวิทยา** *รศ.พญ.สุดา วรรณประสาท และ ศ.ดร.วิจิตรา ทัศนียกุล ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น และหัวหน้าภาควิชาเภสัชวิทยา มหาวิทยาลัยต่าง ๆ ร่วมอภิปราย* *(หัวข้อนี้อาจจะมีการเปลี่ยนแปลง ทางคณะกรรมการจัดงานประชุมจะแจ้งให้ทราบในภายหลัง)*

ML

Dr. Chiravat Sadavongvivad Memorial Lecture

Integrating Genomics Medicine into Healthcare

Prof. Vorasuk Shotelersuk, MD, FABMG

Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Center of Excellence for Medical Genomics, Bangkok 10330, Thailand

Abstract

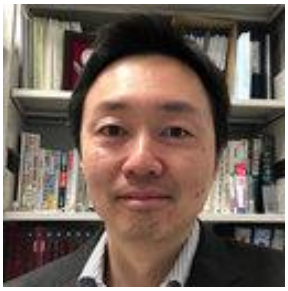
Advances in genetic sequencing technology and computer science have taken molecular biology studies from a level of “genetics” (a single or a few genes) to “genomics” (the whole DNA sequences of an organism). Knowing the meaning and impact of nucleotide variation on health and disease moves the genome from laboratory to medical practice, “genomics medicine”. Combining personalized genomic information with individual behavior and environmental factors generates “precision medicine”. In addition, the rapidly declining sequencing cost has taken the use of genomics for medicine to the level of “precision public health”.

My presentation will highlight the current situation and future global trends of precision medicine. I will also provide updated information on the Genomics Thailand Initiative including policies, directions, infrastructure, work plans, manpower, participation of medical personnel, scientists, and researchers, as well as business opportunities. There are 5 main areas of the research: rare and undiagnosed diseases, cancer, pharmacogenetics, non-communicable disorders and infectious diseases. Importantly, policy on sharing genomics data as well as dimensions about ethical, legal, and social issues (ELSI) will be touched. I will include works of our lab including the discoveries of new human disease genes and their implications. In addition, I will share our real experience in the application of clinical exome and genome sequencing in clinical practice in a Thai context.

With the potential of genomics, medical practice at the level of public health using genetic diversity information has been started. Genomics information is predicted to play more and more roles in health and medicine. Personnel of all fields, including lay persons, are required to be knowledgeable and genomics-literate to be able to understand the genetic and genomic information and its consequences, in order to make right decisions. This will lead to maximum benefits with the least negative impact of genomics.

PL-1

Evaluation of Pharmacokinetics and Efficacy of Immune Checkpoint Inhibitors ~ Lessons from Animal Tumor Models



Assoc. Prof. Hiroto Hatakeyama, Ph.D.

The laboratory of Clinical Pharmacology and Pharmacometrics, Graduate School of Pharmaceutical Sciences, Chiba University, Japan

Abstract

Immune checkpoint inhibitors (ICIs) are currently used in cancer immune therapy. Even though ICIs exhibit superior efficacy and long-term effectiveness, response rate is not high. Translational research (TR) and reverse translational research (rTR) processes are important to elucidate not only mechanisms of diseases but also identify mechanism of actions/adverse reactions of drugs and biomarkers. The pharmacodiscovery cycles of TR/rTR requires information from both basic and clinical sides. Many clinical trials in terms of ICIs are in progress. However, there are a few available information of non-clinical models.

In the clinic, the expression level of PD-L1 has been used as a biomarker for efficacy of Pembrolizumab, a PD-1 monoclonal antibody (mAb). However, the prediction cannot perfectly distinguish responders and non-responders. We evaluated the efficacy of anti-PD-1 mAb in about 20 mouse tumor models. As we expected, the expression of PD-L1 mAb in tumors were not correlated with the efficacy of aPD-1 mAb. This indicates that PD-L1 expression level is not enough to explain responsiveness of tumors to aPD-1 mAbs. We have investigated whether other immune checkpoint molecules can be combined with PD-L1 to improve the prediction of sensitivity to aPD-1 mAb, and compared with human clinical data.

We have also investigated the relationship between pharmacokinetics (PK) and efficacy of ICIs using mouse models. We found that some tumor models were responded to aPD-1 mAb, which indicated that those tumor models were sensitive to PD-1/PD-L1 blockade. However, aPD-L1 mAb filed to suppress tumor growth. We hypothesized the difference in PK between both mAbs caused the difference in the efficacy. We compared PK of mAbs labeled with two radioisotopes to elucidate distribution and degradation in tissues. As a result, aPD-L1 mAb distributed in normal organ rather than tumors and degraded in organs much greater than aPD-1 mAb. Collectively, the poor PK characteristics of aPD-L1 mAb caused lower antitumor activity than of aPD-1 mAb (Kurino T. *J ImmunoTher Cancer*, 2020). Thus, targeting of PD-1 would be more advantageous than that of PD-L1 in terms of PK characteristics.

We will discuss our findings from mouse models that could give answers for clinical questions.

PL-2

Translating Discoveries to Clinical Applications in Treating Asian Lymphoma – From Bench to Bedside

Ong Choon Kiat , Ph.D.

National Cancer Centre Singapore, Singapore

Abstract

Natural Killer/T-cell lymphoma (NKTL) and Monomorphic Epitheliotropic Intestinal T cell lymphoma (MEITL) are very aggressive subtypes of non-Hodgkin's lymphoma with very poor prognosis. The incidence of these diseases is more prevalent in Asia compared to the West. Recent studies have shown that about 50% of relapsed/refractory NKTL response well to PD-1 blockade therapy and little progress has been made for MEITL. I will talk about our effort in discovering novel therapeutic targets for both NKTL and MEITL. I will also describe how we have developed biomarkers of response to treatment and bringing them to clinical applications.

SYM 1-1

Pharmacogenomics of Severe Cutaneous Adverse Drug Reactions and Their Implementation in Healthcare System Policy

Prof. Wichittra Tassaneeyakul, Ph.D.

Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

Severe cutaneous adverse drug reactions (SCARs) including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug reaction with eosinophilia and systemic symptoms (DRESS) are rare adverse drug reactions but life-threatening. Results from our registration data base reveal that the top five most common causative drugs of SCARs in Thailand are carbamazepine, allopurinol, co-trimoxazole, phenytoin and phenobarbital. Genetic factors involved in these drug-induced SCARs in a Thai population, particularly the genetic polymorphism of human leukocyte antigen (HLA) have been identified. These included *HLA-B*15:02* for carbamazepine, *HLA-B*58:01* for allopurinol, *HLA-B*13:01* for co-trimoxazole and *HLA-B*56:02/04* for phenytoin. Universal screening of *HLA-B*15:02* and *HLA-B*58:01* prior prescription of carbamazepine and allopurinol in a Thai population represent good value for the money in terms of preventing SJS/TEN in carbamazepine-treated patients and allopurinol-treated patients. The National Health Security Office has recently approved these pharmacogenetic testing for the Universal Health Coverage. Pathways from bench to policy implementation of these pharmacogenetic tests by NHSO will be discussed.

SYM 1-2

Pharmacogenomics Thailand: Research and Clinical Implementation

Assoc. Prof. Chonlaphat Sukasem, Ph.D.

Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Bangkok 10400, Thailand

Abstract

Several barriers present challenges to implementing pharmacogenomics into practice. This session will provide an overview of the current pharmacogenomics practices and research in Thailand, address the challenges and lessons learned from delivering clinical pharmacogenomic services in Thailand, emphasize the pharmacogenomics implementation issues that must be overcome, and identify current pharmacogenomic initiatives and plans to facilitate clinical implementation of pharmacogenomics in Thailand. Ever since the pharmacogenomics research began in 2004 in Thailand, a multitude of pharmacogenomics variants associated with drug responses have been identified in the Thai population, such as *HLA-B*15:02* for carbamazepine and ox-carbazepine, *HLA-B*58:01* for allopurinol, *HLA-B*13:01* for dapsone and cotrimoxazole, *CYP2B6* variants for efavirenz, *CYP2C9*3* for phenytoin and warfarin, *CYP3A5*3* for tacrolimus, and *UGT1A1*6* and *UGT1A1*28* for irinotecan, etc. The future of pharmacogenomics guided therapy in clinical settings across Thailand appears promising because of the availability of evidence of clinical validity of the pharmacogenomics testing and support for reimbursement of pharmacogenomics testing.

SYM 1-3

Clinical Pharmacogenomics: Application in Transplantation

Asst. Prof. Pajaree Chariyavilaskul, Ph.D.

Clinical Pharmacokinetics and Pharmacogenomics Research Unit, Chulalongkorn University, Bangkok, Thailand

Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract

To date, there are advances in the knowledge in regard to solid organ transplantation in both surgical and medical aspects. In Thailand, kidneys are the major organ which have the highest transplantation rate followed by liver and heart. The national guidelines for matching donors and recipients are readily available. However, donors and recipients who are qualified for transplantation are still suffering from complications and risks of graft rejection. Majority of recipients still require high dose of immunosuppressive agents to maintain their graft.

Our research team are aware of these problems and the plan to improve survival and quality of life of the recipients were implement. A study was conducted in liver transplantation focusing on the mismatch between the pharmacogenetics of donors and recipients and the effects of these differences on levels or dose of immunosuppressive drugs. Preliminary data together with proposed clinical application will be presented.

SYM 2-1

Overcoming Cancer Drug Resistance using the Acquired Vulnerability Screens

Assoc. Prof. Siwanon Jirawatnotai, Ph.D.

Siriraj Center of Research Excellent for Systems Pharmacology and Department of Pharmacology, Faculty of Medicine, Siriraj hospital, Mahidol University, Bangkok, Thailand

Abstract

Cancer drug resistance is at the center of problems for cancer treatment. The resistance may be developed out of chemo-, targeted, as well as immune-based therapies. After each round of resistance to the treatment, patient is left with decreasing chance of treatment. Here, we propose drug screening platforms that based on the acquired vulnerability phenomena, which based on the notion that cancer cell pathways need to reprofile (at DNA, RNA, or protein level) in order to survive (resist) the drug treatment. Thus, the reprofiled cancer may be vulnerable to the new and unrelated treatment. We used the small, FDA-approved cancer drug library, and High-content imaging and Live-cell imaging platforms to screen for acquired vulnerabilities of Gemcitabine/Cisplatin (GEM/CIS)-, and Palbociclib (CDK4 inhibitor)-resistant cholangiocarcinoma (CCA) cells. We found that our platforms were versatile, easy-to-use, and effective. We uncovered acquired vulnerability of GEM/CIS-resistant CCA to LCL161, IAPs inhibitor. In addition, we found that the Palbocilib-resistant CCA were especially hypersensitive to ribosome biogenesis stressors, such as Oxaliplatin, Actinomycin, Phenanthriplatin. At the molecular level, Palbociclib-resistant cells accumulated ribosomal stress, and produced high level of RPL29. The accumulation of RPL29 conferred cell survival under the CDK4 inhibitor. Depletion of RPL29 by siRNAs reversed the Palbociclib resistance.

We also confirmed that combination of the ribosome biogenesis stressor into CDK4 inhibitor was synergistic and can effectively overcome the CDK4 inhibitor resistance, both *in vitro* and *in vivo* xenograft models. Further clinical trial is required to see the effect of the combination in patients.

SYM 2-2

Targetable Fusion Genes in Cancers: The NGS Approach for Precision Diagnosis and Treatment

Asst. Prof. Sarinya Kongpetch, Ph.D.

Department of Pharmacology, Faculty of Medicine,
Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

Fusion genes have been recognized as driver event during tumorigenesis for decades, with efficacies facilitated in clinical diagnosis and targeted therapy. The tumor specific event of fusion genes to neoplastic tissues and their oncogenic functionalities during carcinogenesis making them as promising tools in the battle against cancer. As people studied the BCR-ABL oncogene, they developed a pharmaceutical, imatinib, which directly targeted the ABL kinase in addition to a few other kinases. This now created a predictive test as well as an indicator of therapeutic response.

With advances in sequencing technologies and computational biology, a surge in the identification of fusion genes has been accelerated clinical translation of these internal markers to be biomarkers with clinical utility. High-throughput sequencing technologies, whole-genome, whole-exome and RNA sequencing facilitate the detection of novel fusion in tumors. However, they are impractical with respect to cost and efficiency in the context of clinical molecular diagnostic. Here, we have employed a targeted RNA sequencing approach that requires low RNA input was tested. Anchored multiplex PCR-based enrichment (AMP) was used to rapidly identify a broad range of gene fusions. AMP is one such target-enrichment strategy that significantly increases the sensitivity of gene fusion detection by RNA-Seq, enabling detection of chimeric transcripts with single-molecule resolution.

SYM 2-3

Precision Oncology in Clinical Practice

Asst. Prof. Aumkhae Sookprasert, MD

Department of Medicine, Faculty of Medicine,
Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

การรักษาแบบพุ่งเป้าสำหรับโรคมะเร็งนั้นในปัจจุบันถือว่าเป็นการรักษามาตรฐานชนิดหนึ่งและมีใช้แพร่หลายมานานหลายปีทั้งในทั่วโลกและประเทศไทย การบรรยายในวันนี้จะเน้นให้เห็นถึงการรักษาแบบพุ่งเป้าในมะเร็งที่ถือว่าเป็นมะเร็งที่พบบ่อยที่สุดในประเทศไทยคือ มะเร็งปอด

การรักษามะเร็งปอดระยะแพร่กระจายด้วยยาเคมีบำบัดนั้นถือว่าเป็นการรักษามาตรฐานตั้งแต่ 20 ปีที่แล้ว แต่อย่างไรก็ตามประสิทธิภาพไม่ค่อยน่าจะเป็นที่น่าพอใจนัก เนื่องจากแม้จะถือเป็นมาตรฐาน โอกาสที่ผู้ป่วยจะตอบสนองแบบก้อนยุบนั้นมีไม่เกินร้อยละ 30 ควบคุมโรคได้เฉลี่ยเพียง 4 เดือนและมีค่ากึ่งกลางรอดชีวิตไม่ถึง 1 ปี ที่สำคัญคือเราไม่มีทางรู้เลยว่าคนไข้คนใดจะเป็นคนที่ตอบสนอง แต่ในปัจจุบันเนื่องจากเราทราบว่าผู้ป่วยมะเร็งปอดโดยเฉพาะที่ไม่มีความเสี่ยงเรื่องการสูบบุหรี่ มีความผิดปกติในระดับโมเลกุลหลายชนิดและแต่ละชนิดนั้นมียาพุ่งเป้าที่จำเพาะและได้ผลการรักษาที่ดี ดังนั้นจึงเป็นที่มาของแนวทางการตรวจรักษามะเร็งปอดในปัจจุบันคืออันดับแรกต้องนำชิ้นเนื้อมาตรวจยีนส์ที่ผิดปกติที่จำเพาะ ได้แก่ EGFR mutation, ALK re-arrangement, ROS-1 re-arrangement, RET re-arrangement, BRAFV600E, TRK re-arrangement, MET exon 14 skipping mutation แต่เนื่องจากวิธีการตรวจมีข้อจำกัดเนื่องจากการตรวจ gene re-arrangement นั้นค่อนข้างจำเพาะ และอุบัติการณ์ของความผิดปกติในระดับโมเลกุลส่วนใหญ่คือร้อยละ 90 คือ EGFR mutation และ ALK re-arrangement (โดยทำทางอ้อมคือการย้อมโปรตีน ALK โดยวิธี IHC ซึ่งมีการศึกษายืนยันแล้วว่า correspond กับการตรวจมาตรฐานโดยวิธี break- apart FISH) ดังนั้น รพ.ในประเทศไทยส่วนใหญ่จะตรวจเฉพาะความผิดปกติสองชนิดนี้ก่อน ถ้าไม่พบจึงจะตรวจความผิดปกติอื่นต่อไป

EGFR mutation เป็นความผิดปกติที่พบบ่อยที่สุดถึงร้อยละ 50 ของผู้ป่วยมะเร็งปอดระยะแพร่กระจายในประเทศไทย ตำแหน่งที่พบบ่อยมีสองตำแหน่งคือ EXON19 deletion และ EXON21 ตำแหน่ง L858R ปัจจุบันมียาในกลุ่ม EGFR-TKIs คือยาที่ทำหน้าที่ยับยั้งการทำงานของเอ็นไซม์ tyrosine kinase ส่งผลให้เซลล์มะเร็งไม่สามารถเจริญเติบโตได้ ยาในกลุ่มนี้ในปัจจุบันมี 3 generations โดยตัวที่เป็นมาตรฐานที่ใช้เป็นเวลานานคือกลุ่ม first generations คือ gefitinib และ erlotinib ซึ่งยาทั้งสองตัวมีข้อมูลว่าเปรียบเทียบกับยาเคมีบำบัด ยา EGFR-TKIs มีประสิทธิภาพในแง่การควบคุมโรคดีกว่าอย่างชัดเจนโดยมีอัตราก้อนยุบถึงร้อยละ 70 ระยะเวลาเฉลี่ยในการควบคุมโรค 11 เดือน และค่ากึ่งกลางรอดชีพมากกว่า 2 ปี ซึ่งดีกว่าเคมีบำบัดอย่างมีนัยสำคัญทางสถิติ และที่สำคัญคือยากลุ่มนี้เป็นยารับประทานซึ่งมีความสะดวกและผลข้างเคียงน้อยกว่าเคมีบำบัดด้วย ทำให้คนไข้มีคุณภาพชีวิตดีขึ้น ส่วนบทบาทของsecond generation TKI ได้แก่ afatinib จะมีบทบาทในแง่ของ uncommon mutation ซึ่งไม่มีข้อมูลของกลุ่มผู้ป่วยกลุ่มนี้ และสุดท้าย third generation TKI คือ osimertinib ซึ่งยากลุ่มนี้มีความพิเศษคือนอกเหนือจากสามารถออกฤทธิ์ยับยั้ง common mutations แล้วยังสามารถมีฤทธิ์ยับยั้ง mutation จำเพาะที่เรียกว่า T790M ซึ่งปกติจะเป็น mutation ที่เกิดขึ้นเป็น secondary mutation หลังจากที่ใช้ first generation TKIs แล้วดื้อยา แต่เมื่อนำมาใช้เป็นการรักษาแบบ first line ศึกษาในผู้ป่วยมะเร็งปอดระยะแพร่กระจายที่มี common mutations แล้วเปรียบเทียบระหว่าง first generation TKIs และ third generation TKI (osimertinib) พบว่า osimertinib ให้ระยะเวลาควบคุมโรคนานกว่าอย่างมีนัยสำคัญทางสถิติ 18.9 เดือน เทียบกับ 10.2 เดือน และให้ค่ากึ่งกลางรอดชีวิตที่ยาวนานกว่าอย่างมีนัยสำคัญทางสถิติและถือว่าเป็นยาที่เป็นมาตรฐานใหม่สำหรับคนไข้กลุ่มนี้ในปัจจุบัน

นอกเหนือจากมะเร็งปอดแล้ว การรักษาแบบมุ่งเป้าตอนนี้ได้แพร่หลายและเป็นมาตรฐานในมะเร็งอีกหลายชนิด และถือเป็นความก้าวหน้าที่สำคัญของการรักษามะเร็งทั้งในปัจจุบันและต่อไปในอนาคต

SYM 3-1

Doctor's Concept of Patient Care and the Principles of Personalized Nutrition and/or Personalized Nutraceuticals

Kongkiat Kespechara, M.D.

Bangkok Hospital Pattaya, Chonburi, Thailand

Abstract

In the last decade, the gene sequencing test is become as affordable test for a lay person. In many countries the national genomic project was created even in Thailand, The Genomic Thailand Project which will test 50,000 volunteers to seek for rare diseases and also the new opportunities to offer the new knowledge and the treatment for non-communicable disease such as essential hypertension. The known genetic architecture of blood pressure now comprises > 30 genes, with rare variants resulting in monogenic forms of hypertension or hypotension and > 1,477 common single-nucleotide polymorphisms (SNPs) being associated with the blood pressure phenotype, Citterio *et.al*, show that genetics may guide drug treatment for primary hypertension in Caucasians but not in Chinese. We also found benefit of using genomics for cancer even in stroke prevention with anticoagulant and in various usage as pharmacy-genomics.

In the new approach, preventive medicine is the future trend for health care. With the challenge of specific usage of supplement to enrich health, the nutraceuticals are the halfway between pharmacy and supplement. To use nutraceutical as the specific aims to achieve the preventive outcomes with genomic information still need more data for practices. The personalized nutraceuticals define as Gibney *et al*. describe it as an approach that "assists individuals in achieving a lasting dietary behaviour change that is beneficial for health. Personalised nutrition can be applied in two broad areas: firstly, for the dietary management of people with specific diseases or who need special nutritional support—for example, in pregnancy or old age, and, secondly, for the development of more effective interventions for improving public health. It has traditionally focused on maximizing the benefits and reducing the adverse effects of dietary changes for the individual.

The trend towards personalization is the result of: firstly, nutrition research that provides a better understanding of how diet affects health; secondly, new technology that enables better and continuous measurements of markers of individual health and fitness; and thirdly, new analytical tools that interpret this flow of data and transform it into user friendly practical information.

Those can be the keys to give us clear understanding about the benefit and practical use of personalized nutritions/nutraceuticals in clinical practices.

SYM 3-2

Nutraceuticals Targeting Gut Microbiota for Precision Medicine

Asst. Prof. Siam Popluechai, Ph.D.

Gut Microbiome Research Group, Mae Fah Luang University, Muang, Chiang Rai, Thailand

School of Science, Mae Fah Luang University, Muang, Chiang Rai, Thailand

Abstract

Nutraceuticals are defined as any substance that is a food or part of a food and provides medical or health benefits, including the prevention and treatment of diseases. The human gut microbiota is the collection of eukaryotic and prokaryotic microorganisms forming a complex ecosystem in the intestinal tract. Many studies demonstrated the pivotal roles of gut microbiota in health and diseases. Recent studies showed that many nutraceuticals change the composition of the microbiota and can interfere with the health status of the host. However, the extent of modification varies from person to person due to the variation of the gut microbiota. In this study, I will introduce (1) the effects of the gut microbiota on the efficacy and toxicity of nutraceutical (2) the variability of gut microbiota which leads to the variation of nutraceuticals response (3) the biomarkers used for patient treatment decision of nutraceuticals.

SYM 3-3

Rice as an Example of a Personalized Lifestyle Medicine for NCDs

Assoc. Prof. Jintana Sattayasai, Ph.D.

Department of Pharmacology, Faculty of Medicine,
Khon Kaen University, Khon Kaen 40002, Thailand

Abstract

Nowadays, the burden of non-communicable diseases (NCDs) is rising and many nutraceuticals are being used to help relieving and/or preventing of many NCDs. Rice, the seed of the grass species *Oryza sativa* (Asian rice), is a cereal grain which is the most widely consumed staple food especially in Asia. Rice is reported to have nearly all types of nutrients including macro- and micro-nutrients and enriched with many biologically active compounds, including polyphenols, vitamin E, polyunsaturated fatty acids and phytosterols especially gamma-oryzanol. Studying the effects of unpolished dark purple glutinous rice (Luem Pua Rice, LP) and gamma-oryzanol-enriched rice bran oil (GRBO) in cell culture, animals, and humans reveal the high antioxidant activities with many health benefits especially for chronic NCDs such as cognitive impairment, hypertension, dyslipidemia, diabetes and inflammatory bowel disease (IBD). In addition to the antioxidative effect, LP rice possess muscarinic-like action and reduces the gut microbiota dysbiosis and GRBO increases adiponectin release without effect on PPAR-g expression, which might strengthen gut-brain health and be responsible for the positive effects on memory-impairment, IBD and many aspects of NCDs. However, as pathogenesis of many disorders, especially NCDs, are multifactorial, LP rice and GRBO are not the ones for all diseases or health problems but could be considered as a “Personalized Lifestyle Medicine” for NCDs.

SYM 4-1

Structural Virology and Immunology in SARS-CoV-2

Asst. Prof. Puey Ounjai, Ph.D.

Department of Biology, Faculty of Science,
Mahidol University, Bangkok 10400, Thailand

Abstract

The global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has tremendous effects on almost every aspects of life. This session will provide an overview of the current understanding on structural biology of the SARS-CoV-2 infection with an emphasis on the technology that allow the structural determination of viral components at molecular details as well as the recent visualization technology that enable mechanistic investigation of the SARS-CoV-2 infection in their physiological condition. The overview of the complex interaction between the virus and host immune system will be elaborated including the mutation of viral spike proteins that allow the virus to evade immune detection and neutralization. The abnormal elevation of cytokine level proposed to be associated with clinical complication, so-called cytokine storms will also be discussed. Recent development of alternative antibody-based biologics against SARS-CoV-2 that can be utilized to alleviate and prevent severe infection will also be further scrutinized. The insights offered in this talk should lay a foundation for further development of novel detection and therapeutic strategies against SARS-CoV-2.

SYM 4-2

Pharmacological Treatment of COVID-19

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is the defining global health crisis since cases were first reported in December 2019. Patients with COVID-19 present with a spectrum of clinical manifestations from asymptomatic or mild, self-limited constitutional symptoms to a hyperinflammatory state (cytokine storm) followed by acute respiratory distress syndrome and death. There are two major processes that are thought to drive the pathogenesis of COVID-19. In the early infection stage, the disease is primarily driven by replication of SARS-CoV-2. Later stages of infection, the disease is driven by an exaggerated immune/inflammatory response to the virus that leads to tissue damage. Based on this understanding, it is anticipated that antiviral therapies would have the greatest effect early in the course of disease, while immunosuppressive or anti-inflammatory therapies are likely to be more beneficial in the later stages of COVID-19. This session will provide the information of the potential and available pharmacological treatments for COVID-19, focusing on antiviral, immunomodulatory and/or anti-inflammatory agents.

SYM 4-3

Current Status of COVID-19 Vaccine Development

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Abstract

From the COVID-19 pandemic, vaccine developers and researchers around the world have put their highest attempt to develop the vaccine against Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in order to serve the country and in time for pandemics. The WHO has followed-up and reported the vaccine development landscape from the vaccine developers around the world. With modern technologies, the development of COVID-19 vaccines have transformed the development of all vaccines in the past, which normally takes 10-15 years each to develop just one vaccine. The new COVID-19 vaccines have been developed with a much shorter time that the first approved COVID-19 vaccine for emergency use, the mRNA vaccine, took less than a year. One of the key important factors is because scientists and vaccine developers have been experiencing with the development of vaccine against prior known coronavirus causing SARS (Severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome). At present, there are at least 12 vaccines which have already been approved, with more than 80 candidate vaccines are still in the clinical trials and more than 180 others candidate vaccines are being test in pre-clinical studies. In terms of technology, the 12 vaccines that have already been approved include (1) 2 mRNA nucleotide-based vaccines, which use the genetic materials or the mRNA of the spike protein of coronavirus as the vaccine antigen. This mRNA will be expressed and translated to spike protein of coronavirus in the human body, (2) 4 inactivated viral-based vaccines which produced from the inactivated coronavirus, (3) 4 viral vector-based vaccines which use other attenuated viruses i.e. human adenovirus (Ad5, Ad26), chimpanzee adenovirus (ChAdOx1) to bring in the gene of coronavirus spike protein into the human body and (4) 2 protein–based vaccines which use a part of spike protein (i.e. receptor binding domain of the spike protein) or the whole part of spike protein (only the whole S protein with or without the viral envelope) of coronavirus as vaccine antigen.

Apart from vaccine development, there are some other interesting research projects on uses of these vaccines i.e. Covid-19 Com-COV study for the uses of different vaccines in combinations and to the use of the two-dose vaccine at longer interval to facilitate vaccine administration etc.

In summary, COVID-19 vaccine development is just at the beginning of the success and only immunization in large population will help generating herd immunity. There are also some other challenges for us to keep up with the research to see whether the vaccines are protective against the variant coronavirus strains as new strains developed, or to learn whether the immune response persisted, otherwise we might end up with having to re-vaccinate every year with one or more strains of this vaccine.

SYM 5-1

Modulation of Metabolic Cellular Sensor as a Strategy in Tumor Suppression

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Abstract

Tumors have a metabolism that is different from those found in healthy tissues. Typically, altered metabolism in cancer cells is pivotal for tumor growth, particularly by providing energy, reducing equivalents and building blocks while several oxidative metabolites exert a signaling purpose promoting tumor growth and progression. During the past decades, substantial research has been done which clearly suggests metabolic reprogramming and dysregulated oxidative stress in cancer are linked to the development of treatment resistance in several ways. Recent studies have demonstrated that AMPK is a metabolic sensor, whereas activation of AMPK has been found to oppose tumor progression in several cancer types. On the other hand, dysregulated oxidative stress is critical for promoting tumorigenesis and failure of treatment, ROS modulator has emerged as an alternative cancer therapy. It is logical to assume that modulation of altered metabolism and dysregulated oxidative stress may aid in the precision targeting of altered behaviors with a focus on combination strategy to suppress tumor progression and enhance the treatment.

SYM 5-2

Development of a Precision Approach against Skin Aging

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Abstract

The aging population in industrialized countries including Thailand continues to rise and age-associated skin diseases have also been recognized as an important health issue that should be integrated into the global strategy on aging. Both intrinsic and extrinsic factors are accountable for skin aging. While intrinsic aging is generally determined by genetic influences, environmental factor, in particular ultraviolet radiation (UVR), can significantly influence and superimpose the changes in chronological aging that lead to premature aging or photoaging. Since people's individual variations in genes, environment (UVR and pollution) and lifestyle also accountable for premature aging should be taken into account, the development of a precision approach against skin aging is essential to optimize the preventive and therapeutic interventions. UVR plays a role in the photodamage through triggering various biological responses of skin cells including apoptosis, oxidative stress, DNA damage, inflammation and melanogenesis. Our *in vitro* and *in vivo* studies have demonstrated that UVA could induce photoaging of cultured skin cells and mouse skin via the generation of reactive oxygen species (ROS) involved in upregulating matrix metalloproteinase-1 (MMP-1 or collagenase-1) responsible for collagen destruction, the crucial hallmark of photoaged skin. Furthermore, homeostasis of the skin is complex and regulated by various factors (such as α-melanocyte-stimulating hormone; α-MSH) secreted by epidermal keratinocytes for maintenance of melanocyte homeostasis. We have reported the photoprotective roles of nuclear factor erythroid 2-related factor 2 (Nrf2), an important transcription factor controlling antioxidant responses, in UVR-mediated skin photodamage and photooxidative stress via modulation of signaling pathways (such as MAPK signaling) involved in upregulation of MMP-1. In this respect, indirect or direct targeting of Nrf2-dependent antioxidant defenses to combat oxidative stress and damage may represent a promising pharmacological strategy for the prevention and inhibition of skin photoaging. Furthermore, previous genome-wide association studies (GWASs) suggested an association of MC1R (melanocortin-1 receptor), a pigmentation gene, in youthful looks. We also identified that Nrf2 could modulate the protective effect of keratinocytes-derived paracrine factor α-MSH, a major agonist for MC1R, on the stress response of melanocytes to UVB. Hence, identification of Nrf2-regulated cytoprotective genes and candidate paracrine genes in response to UVR using transcriptome approach could provide insights into potential biomarkers of the skin susceptibility to photodamage that might lead to the future development of precision approaches for preventing the environmental impacts on skin health. Further investigation is warranted concerning a systematic characterization of genetic differentiation and/or variation across subpopulations associated with chronological age and premature (photo) aging.

SYM 5-3

Ferroptosis and Development of Lipid Radical Inhibitors for Diseases with Lipid Oxidation

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Abstract

Ferroptosis is a form of regulated cell death that is mainly mediated by iron-dependent lipid peroxidation. It is distinct from apoptosis and other forms of cell death. Ferroptosis is characterized by accumulation of lipid hydroperoxides which due to impairment of glutathione peroxidase 4 (GPX4) activity. Recent studies have proven the association of ferroptosis with pathological process of several diseases such as cancer, neurodegenerative diseases and cardiovascular diseases. Therefore, the drug discovery and development has moved toward targeting the processes of ferroptosis. There are two classes of ferroptosis inhibitors, class I inhibitors of iron accumulation and iron chelators, and class II inhibitors of lipid peroxidation including antioxidants and lipid radical traps. Ferrostatin-1 (Fer-1) and liproxstatin-1 (lip-1) are well-known potent ferroptosis inhibitors that act as lipid alkoxyl radical scavengers. Moreover, there are several compounds both natural and synthetic compound are investigated. Here, the development of lipid radical scavengers as ferroptosis inhibitors will be introduced. In addition, novel technique for screening lipid radical traps will be discussed.

ABSTRACTS

A001

A Randomized Placebo-controlled Phase I Clinical Trial to Evaluate the Immunomodulatory Activities of *Atractylodes lancea* (Thunb) DC in Healthy Thai Subjects

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ABSTRACT

Introduction: *Atractylodes lancea* (Thunb) DC. (AL) and bioactive compounds β-eudesmol and atractylodin have been demonstrated in the *in vitro* and *in vivo* studies for their potential clinical use in cholangiocarcinoma.

Objectives: The study was a randomized, double-blinded, placebo-controlled phase I clinical trial to evaluate the immunomodulatory effect of AL in human subjects.

Methods: The modulatory effects of AL and β-eudesmol and atractylodin on TNFα and IL6 expression in the isolated PBMCs in culture were measured using real-time PCR. In the human study, blood samples were collected from forty-eight healthy subjects following oral administration of a single or multiple dosing of capsule formulation of the standardized AL extract or placebo. Serum cytokine profiles, lymphocyte subpopulations (B lymphocytes, $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T-helper lymphocytes, and NK cells), and cytotoxic activity of PBMCs against the cholangiocarcinoma cell line CL-6 were evaluated using cytometric bead array (CBA) with flow cytometry analysis. Approval of the study protocol was obtained from the Ethics Committee, Thammasat University.

Results: AL extract at almost all concentrations significantly inhibited both TNFα and IL6 expression in Con A-mediated inflammation in PBMCs in culture. β-Eudesmol at all concentrations significantly inhibited only IL6 expression. Atractylodin at the lowest concentration significantly inhibited the expression of both cytokines, while the highest concentration significantly inhibited only IL6 expression**.** The administration of AL at a single oral dose of 1,000 mg decreased IFNγ and IL10 and increased B cells while significantly increased NK and $CD4^+$ and $CD8^+$ cells. A trend of increasing (compared with placebo) cytotoxic activity of PBMCs at 24 h after dosing was observed. AL given at multiple dosing of 1,000 mg for 21 days tended to decrease the production of all cytokines while significantly inhibited IL17A production at 24 h of dosing. In addition, a significant increase in $CD4^+$ and $CD8^+$ cells was observed. A trend of increase in the cytotoxic activity of PBMCs was observed at 24 h but terminated at 48 h of dosing.

Conclusions: The results confirm the immunomodulatory activity of AL in humans. This activity, in complementary with the direct action of AL on inducing cholangiocarcinoma cell apoptosis, suggests its potential role for CCA control.

Acknowledgement: *The study was supported by Thammasat University (Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma) and the National Research Council of Thailand. Kesara Na-Bangchang is supported by the National Research Council of Thailand under the Research Team Promotion grant (grant number 820/2563). Inthuon Kulma is supported by Thailand Research Fund under the Royal Golden Jubilee PhD Program (grant number PHD/0096/2560).*

A002

In Vitro, Antioxidant Properties of Black Pepper Extract

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ABSTRACT

Introduction: Ultraviolet B (UVB) radiation from the sunlight can penetrate mainly the epidermis. UVB initiates detrimental photochemical reactions and causes skin inflammation. Black pepper containing piperine exhibits antioxidant, antitumor, anti-inflammatory, antibacterial, and antifungal capacities.

Objective: We investigated the antioxidant and protective effects of methanol extract of black pepper on UVB-irradiated human keratinocytes.

Methods: Total phenolic and flavonoid contents were evaluated by Folin-ciocalteu and aluminium chloride colorimetric assays, respectively. The photo-protective and antioxidant of the extract were further investigated by resazurin assay and Griess assay, respectively.

Results: The total phenolic and flavonoid contents were presented in the extract. Moreover, pre-incubation of keratinocytes with the extract for 1 hour followed by UVB irradiation significantly increased cell survival. Moreover, the extract significantly decreased the levels of nitric oxide (NO) production in a concentration-dependent manner. The extract at the studied concentration was not toxic to keratinocytes.

Conclusion: The results showed that the extract protected cell death which might be related to the reduction of NO generation by UVB-irradiation. The phenolic and flavonoid compounds in the extract may be responsible for antioxidant activity. Thus, the extract may be developed as a protective agent for the prevention of UVB-induced skin damage. Further investigation should elucidate the anti-inflammatory mechanism of the extract.

Acknowledgement: *Faculty of Medicine, Srinakharinwirot University and Graduated School of Srinakharinwirot University*.

A003

The Inhibitory Effect of Oxyresveratrol on NO Production and COX-2 Protein in LPS-activated Microglial Cells

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ABSTRACT

Introduction: Microglia respond to detrimental stimuli which lead to the inflammatory response-mediated neuroinflammation and contribute to neurodegeneration. Therefore, the inhibition of microglial activation-mediated neuroinflammation by anti-inflammatory and antioxidant agent appears an effective therapeutic strategy for alleviating neurodegenerative diseases. Oxyresveratrol, a stilbenoid is mostly found in the heartwood of *Artocarpus lakoocha* and exerts antioxidant capacity as well as anti-inflammatory effect.

Aim: This study investigated whether oxyresveratrol has antioxidant and anti-inflammatory effect in lipopolysaccharide (LPS)-activated BV2 microglia.

Methods: Cytotoxicity of oxyresveratrol was evaluated, and its protective effect in LPS-activated microglia was examined using resazurin cell viability assay. The antioxidant and inhibitory effects of oxyresveratrol on cyclooxygenase-2 (COX-2) protein expression in LPS-activated microglia were investigated by Griess assay and western blot analysis, respectively.

Results: Oxyresveratrol significantly inhibited the NO production and COX-2 protein expression in LPS-activated microglia. The studied concentration of Oxyresveratrol was not toxic to the cells. The results indicated that oxyresveratrol exerted anti-inflammatory properties by suppressing the generation of NO production in LPS-stimulated microglia.

Conclusion: Anti-inflammatory property of oxyresveratrol may be useful for treating the inflammatory response of microglial activation-induced neurodegeneration. Further investigation needs to evaluate the other possible mechanisms involved.

Acknowledgement: *Faculty of Medicine, Srinakharinwirot University and Graduated School of Srinakharinwirot University.*

A004

N-methylatalaphylline from *Atalantia monophylla* Inhibits Colon Adenocarcinoma Cell Proliferation, Migration and Invasion through Regulating JNK Pathway

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ABSTRACT

Introduction: Colon cancer is one of the most common cancers found in Thailand, manifested by abnormal cell growth, losses of cell apoptosis, and spreading to other organs. Thus, the inhibition of cell growth, migration and invasion of cancer cells are the goals of colon cancer treatment.

Objectives: To investigate the inhibitory activity and molecular mechanism of *N*-methylatalaphylline on cell growth, migration and invasion of human colorectal adenocarcinoma cells (HT-29).

Methods: *N*-methylatalaphylline from *Atalantia monophylla* was investigated for cell cytotoxicity by using MTT assay. The cell morphology was observed by using phase contrast microscope. Effects of *N*-methylatalaphylline on cell migration and cell invasion were evaluated by using Transwell migration assay and Transwell invasion assay. The molecular mechanism of *N*-methylatalaphylline on signaling proteins involving cell proliferation, migration and invasion pathways was investigated by immunoblotting analysis. Doxorubicin and cycloheximide were used as the positive control.

Results: *N*-methylatalaphylline at 100 µM significantly induced cell death in HT-29 cells ($p<0.05$) which was correlated with cell morphological changes. A study using JNK-siRNA transfected cells, the Western blot results showed that *N*-methylatalaphylline inhibited HT-29 proliferation in association with increased cleavage of caspase-3 through JNK pathway. Furthermore, *N*-methylatalaphylline suppressed migration and invasion of HT-29 cells in a concentration-dependent manner by inhibiting matrix metalloproteinase-9 (MMP-9) through JNK pathway.

Conclusions: *N*-methylatalaphylline inhibited cell proliferation, migration and invasion of HT-29 cell through JNK pathway.

Acknowledgement: *This work was supported by grant-in-aids from Faculty of Pharmaceutical sciences, Ubon Ratchathani University, Thailand.*

A005

Nordentatin Inhibits Neuroblastoma Cell Proliferation and Migration through Regulating GSK3 Pathway

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ABSTRACT

Introduction: Cancer is one of the major health problems in Thailand, caused by an abnormal cell change leading to uncontrolled cell growth. The specific characteristics of cancer cells, including the loss of apoptotic control and the ability to migrate and invade the surrounding tissue, resulting in cancer cell metastasis to other parts of the body. Thus, inhibition of proliferation, migration and invasion of cancer cells is the principal goal of the treatment of cancer.

Objectives: To investigate the inhibitory activity of nordentatin on proliferation and migration of human neuroblastoma cell (SH-SY5Y).

Methods: Nordentatin, a coumarin based compounds, was an active constituent derived from the root bark of "Song-fa" (*Clausena harmandiana*). The compound was evaluated for cell cytotoxicity by using MTT assay. The effects of nordentatin on cell migration were investigated by Transwell migration assay. Furthermore, the effects of nordentatin on proliferation, migration and apoptosis in SH-SY5Y cells were investigated by western blot analysis.

Results: Nordentatin at 100 µM showed significant cytotoxicity at 24, 48, and 72 h toward SH-SY5Y cells, compared to the control group ($p<0.01$). Moreover, nordentatin inhibited SH-SY5Y cells proliferation by inhibiting antiapoptotic protein (Mcl-1), leading to the activation of caspase-3. Migration assay showed that nordentatin inhibited cancer cell migration by inhibiting a migrating protein, MMP-9, through GSK-3 pathway as compared with cell treated with GSK inhibitor.

Conclusions: Nordentatin inhibited the proliferation and migration of neuroblastoma cells through GSK-3 pathway.

Acknowledgement: *This work was supported by Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand.*

A006

Carbazole Derivative from *Clausena harmandiana* Inhibits Neuroblastoma Cells through Down-regulating ERK Pathway

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ABSTRACT

Introduction: Cancer is a disease with uncontrolled cell growth and apoptosis, leading to increased cell proliferation and the ability of migration or invasion. Currently, most chemotherapy plays an important role in cancer treatment. Thus, many studies are now focusing on developing a new effective agent for cancer treatment**.**

Objectives: The present study investigates the effect of a carbazole derivative from *Clausena harmandiana* on proliferation, migration and invasion of the neuroblastoma cell line (SH-SY5Y)

Methods: The carbazole derivative (P6) was used to determine its inhibitory effect on SH-SY5Y cells for proliferation by MTT assay, cell migration by Transwell migration assay and cell invasion by Transwell invasion assay. The molecular mechanisms of the carbazole derivative on signaling proteins involved in apoptosis, migration and invasion was studied using western blot analysis.

Results: The effect of carbazole P6 showed a significant antiproliferation at a concentration of 100 µM with the cell viability of 6.25 ± 0.59% at 24 hours. Furthermore, P6 at a concentration of 10 µM showed a significant inhibitory effect on cell migration and invasion ($p<0.05$) in SH-SY5Y cell line. Western blot results showed that P6 inhibited SH-SY5Y cell proliferation by suppression of the expression of anti-apoptotic proteins (Bcl-2, XIAP, Bid) and induction of cleaved caspase-3 through down-regulating ERK pathway. In addition, P6 inhibited the expression of MMP-9 which is a signaling protein related to migration of cancer cells through ERK pathway (comparing with cell treated with ERK inhibitor).

Conclusions: The carbazole derivative, P6 showed inhibitory activity on cell proliferation migration and invasion and induction of apoptosis by down-regulation of ERK pathway in neuroblastoma SH-SY5Y cells.

Acknowledgement: *This work was supported by the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand.*

A007

Clinical Pharmacokinetics and Human Lipidomics of Standardized *Centella asiatica* Extract Orally Administered in Healthy Volunteers

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ABSTRACT

Introduction: A standardized extract of *Centella asiatica* (ECa 233) was developed as an enhanced dissolution capsule and used in clinical pharmacokinetic studies. The test formulation was investigated for its potential to improve lipid metabolic profile in human.

Objectives: The objectives of this study were (1) to investigate the dissolution of the modified capsule and its pharmacokinetic changes and (2) to study the alteration of human lipidomics using MS-based metabolomics approach.

Methods: This study determined dissolution profiles following the United States Pharmacopeia and evaluated the pharmacokinetics and lipidomics of ECa 233 formulation throughout a phase I clinical trial in six healthy volunteers. Each participant received a single 250-mg dose of ECa 233 under a fasting condition. The study protocol has been approved by the Institutional Review Board of Chula Clinical Research Center, Chulalongkorn University (IRB number: 479/61, TCTR20180922001).

Results: The modified formulation of ECa 233 was developed by adding a surfactant to increase the solubility of the poorly water-soluble substances. Triterpene derivatives in ECa 233 were dissolved more than 80% in 45 minutes. This improved formulation was found to expand the pharmacokinetics and extent of parent compounds (madecassoside and asiaticoside). In addition, greater systemic exposure was seen in their respective metabolites (madecassic acid and asiatic acid). Significant changes of lipidome were detected in levels of phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs). Moreover, lysophosphatidylcholines (lysoPCs) and choline were higher after receiving ECa 233.

Conclusions: This finding suggests that the modified ECa 233 possesses enhanced pharmacokinetics due to improved dissolution. Oral administration of ECa 233 could affect plasma lipidomics through alteration of lipid metabolites between pre- and post-dose. The increasing level of lysophospholipid and choline in plasma indicate that ECa 233 may improve cognitive function via this adjustment of the human lipidome.

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A008

MALDI Imaging of Distribution of Atractylodin-loaded PLGA Nanoparticles in Mice Liver

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ABSTRACT

Introduction: Atractylodin, the active compound found in *Atractylodes lancea* (Thunb.) DC. rhizome, has been shown to exhibit potent anticancer activity against cholangiocarcinoma *in vitro*. However, the compound is insoluble in water that could limit its membrane penetration into the liver target organ.

Objectives: The study aimed to develop the atractylodin-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles (ATD-NP) as a drug delivery system to improve the aqueous solubility and, thus, membrane penetration of the compound.

Methods: ATD-NP was developed by the solvent displacement method. The particle size, size distribution, charge and encapsulation efficiency of ATD-NP were determined. Free atractylodin and ATD-NP were administered orally *via* gastric lavage to mice at the dose of 100 mg/kg body weight, and the livers were collected after four hours of administration. The liver samples were sliced, and the biodistribution of atractylodin in the free and ATD-NP forms was analyzed using MALDI imaging mass spectrometry.

Results: The size of the ATD-NP was approximately 200 nm in diameter with a narrow size distribution (0.112 to 0.154) and the surface charge lower than -30 mV. The TEM (Transmission Electron Microscopy) analysis revealed the spherical shape of the nanoparticles. The encapsulation efficiency was 80%. The distribution of the compound in mice liver was 5.4 times higher in ATD-NP compared to unformulated atractylodin.

Conclusions: ATD-NP significantly increased the biodistribution of atractylodin to the liver of mice, suggesting PLGA-NP as a promising delivery system for atractylodin in cholangiocarcinoma.

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A009

Physiologically Based Pharmacokinetic Modeling of CYP450-mediated Erlotinib Drug Interaction with Thai Herbal Extracts

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ABSTRACT

Introduction: Erlotinib is an inhibitor of the epidermal growth factor receptor tyrosine kinase used in the treatment of non-small cell lung cancer and pancreatic cancer. It is metabolized primarily by cytochrome P450 3A4 (CYP3A4). Concomitant use of erlotinib and Thai herbal medicine may result in herb-drug interaction by inhibiting CYP enzymes, leading to the changes in the blood concentration of erlotinib.

Objectives: This study aimed to develop and verified herb-erlotinib interaction using a SimCYP®-based physiologically based pharmacokinetic (PBPK) model. A PBPK model was optimized in SimCYP® for erlotinib as a CYP substrate, based on published *in vitro* and clinical data.

Methods: This model was verified to replicate the magnitude of drug-drug interaction with ketoconazole, a well-known CYP3A4 inhibitor. Herb-erlotinib interactions were predicted with *Andrographis paniculata* (*A. paniculata*), *Curcuma zedoaria* (*C. zedoaria*), *Ganoderma lucidum* (*G. lucidum*), *Murdannia loriformis* (*M. loriformis*), *Smilax glabra* (*S. glabra*) and *Ventilago denticulata* (*V. denticulata*) using the half maximal inhibitory concentration (IC_{50}) values.

Results: The erlotinib SimCYP® model predicted the magnitude of erlotinib AUC and C_{max} values increased by 1.3-1.5 and 1.2-1.4 folds when co-administered with *C. zedoaria*. However, the PBPK simulations of erlotinib with IC_{50} values of *G. lucidum*, *M. loriformis*, *S. glabra* and *V. denticulata* did not show significant changes in pharmacokinetic profiles of erlotinib.

Conclusions: Consequently, patients should be aware of alteration in erlotinib exposure when the drug is concurrently used with Thai herbal medicine.

Acknowledgement: *This study was supported by a research fund for DPST graduate with first placement and Faculty of Science, Mahidol University. We acknowledge the Center of Excellence on Environmental Health and Toxicology (EHT) for providing the equipment.*

A010

Superior Pharmacokinetic Profiles of Madecassoside and Asiaticoside in a Standardized Extract of *Centella asiatica* with Better Water Solubility in Beagle Dogs

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ABSTRACT

Introduction: *Centella asiatica* (Linn.) has been used in alternative medicine to treat many diseases or lesions with good efficacy and safety. ECa 233 is a commercial, standardized *C. asiatica* extract which is sparingly soluble, and thus there is poor absorption after oral administration. Recently, Centell-S was developed and provided by Siam Herbal Innovation Co, which has similar components to ECa 233 and is freely soluble in water. Centell-S may therefore have a superior pharmacokinetic profile compared with ECa 233, especially regarding absorption.

Objectives: The present study investigated the pharmacokinetic profile of active triterpenoids in Centell-S and ECa 233, using beagle dogs as an animal model.

Methods: The concentrations of major bioactive triterpenoids, including madecassoside, asiaticoside, madecassic acid, and asiatic acid, in biological samples were measured by liquid chromatography–tandem mass spectrometry.

Results: Oral administration of Centell-S 10–20 mg/kg generated approximately two-fold higher plasma levels of both madecassoside and asiaticoside compared with oral administration of equivalent doses of ECa 233. In addition, there was accumulation of triterpenoid glycosides after multiple oral administrations of Centell-S for 7 days, while triterpenic acids showed little tendency for accumulation.

Conclusions: The increase of bioavailability of Centell-S is likely resulted from the formulation development to improve water solubility and that is correlated with the plasma concentration of madecassoside and asiaticoside which showed approximately twofold higher than the ECa 233.

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A011

Engineered T Cells Targeting B7H3 Antigen for the Treatment of Common Cancers

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ABSTRACT

Introduction: Chimeric antigen receptor (CAR) is a synthetic receptor that redirects T cells to target surface antigens of tumor cells. CAR T cell therapy has shown impressive clinical response in the treatment of hematologic malignancies. B7 homolog 3 (B7H3) is an immunoregulatory molecule that overexpresses various cancer types and has become of interest as a therapeutic target in CAR T cells therapy.

Objectives: To investigate the specificity of B7H3-targeted CAR (B7H3 CAR) T cells on common cancers in Thailand.

Methods: Five cancer types, including acute lymphoblastic leukemia (NALM-6), glioblastoma (LN229), breast cancer (MDA-MB-231), pancreatic cancer (Capan-2) and cholangiocarcinoma (HuCCT-1), were screened for B7H3 expression. The B7H3 CAR T cells were composed of single chain variable fragment (scFv) of anti-B7H3 antibody, signaling domain of T cell receptor and intracellular domain of the co-stimulatory receptor. This study determined the *in vitro* cytotoxicity of B7H3 CAR T cells on various solid tumors.

Results: B7H3 was expressed on epithelial cancer cell lines including LN229, MDA-MB-231, Capan-2 and HuCCT-1 in a different level ranging from 28.50-97.90%, but not on blood cancer cells (NALM-6). Using retroviral vector, the transduction efficiency of B7H3 CAR T cell was more than 30%. After co-culturing B7H3 CAR T cells with tumor cells for 48 hours, B7H3 CAR T cells specifically lysed the tumor cells expressing B7H3 in a dose-dependent manner.

Conclusions: B7H3 CAR T cells can be generated and applied for common cancer in Thailand expressing B7H3.

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A012

Improving Anti-tumor Efficacy of MUC1 Chimeric Antigen Receptor (CAR) T Cells for the Treatment of Breast Cancer

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ABSTRACT

Introduction: Treatment with chimeric antigen receptor (CAR) T cells faces challenges in solid tumor due to the immunosuppressive microenvironment. In breast cancer, Mucin-1 (MUC1) is over-expressed in the form of hypoglycosylation, which can be used as a target for cellular immunotherapy. However, the overexpression of PD1 ligand may render T cell exhaustion and dysfunction.

Objectives: To develop CAR T cell targeting MUC1 on breast cancer and enhance the efficacy of CAR MUC1 T cell by engineering self-release PD1 blockade.

Methods: We generated CAR MUC1 T cell (MUC1.41BBz) and CAR MUC1 secreting anti-PD1 scFv (MUC1.PD1) using retroviral based system. To study the effector functions between MUC1.41BBz and MUC1.PD1 CAR T cells, we determined cellular composition and cytotoxicity.

Results: We successfully generated CAR T cells targeting MUC1 that is capable of self-release anti-PD1 scFvs. Comparing with MUC1.41BBz, MUC1.PD1 had no significant differences in the level of CAR expression, T cell expansion and cellular composition (Major subset was CD3+ T cell with a higher percentage of CD8+ than CD4+ T cells). In addition, MUC1.PD1 CAR T cells exhibit superior cytotoxic function against breast cancer cells expressing a high level of PD-1 ligand.

Conclusions: Engineering CAR T cells to secrete immune checkpoint blockade can enhance the function of CAR T cells.

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A013

Evaluation of Cytotoxic and Anti-metastatic Effects of Cucurbitacin B on Cholangiocarcinoma Cells

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ABSTRACT

Introduction: Cholangiocarcinoma (CCA) is a malignant tumor arising from biliary epithelium tract of the bile duct system, which has a very poor prognosis. Phytochemical agents with anti-CCA activities using alone or in combination with current chemotherapeutic drugs are still required for CCA treatment to achieve higher efficacy and improvement of the quality of life of patients.

Objectives: To investigate the effects of cucurbitacin B (CuB), a natural tricyclic triterpenoid, on cell viability, migratory and invasive abilities, and the expression of metastasis-related proteins of CCA cells.

Methods: CCA cells, KKU-452, were used in the study. Cytotoxicity was assessed by sulforhodamine B colorimetric method. Cell migration was analyzed by wound healing assay, and invasion ability of the cells was quantified by Transwell Boyden chamber. The expression of metastasis-associated proteins including FAK, MMP-9 and VEGF were determined by Western blot analysis.

Results: CuB exerted a potent cytotoxicity effect against CCA cells with an IC_{50} value of 0.66 ± 0.02 µM at 24 h. The combination of CuB with cisplatin potentiated the cytotoxic effect against KKU-452 cells when compared with cisplatin alone. Additionally, CuB could inhibit migratory and invasive abilities of CCA cells. Detailed molecular analyses demonstrated that the anti-metastasis effect of the compound was associated with the suppression of metastasis-related protein expressions in CCA cells.

Conclusions: CuB exhibits effective anti-CCA activities by suppressing growth and metastasis behaviors of CCA cells. These findings support the potential of CuB to be developed as a novel therapeutic agent for CCA therapy.

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A014

Molecular Surveillance of *Plasmodium falciparum Multidrug Resistance 1* (*Pfmdr1*) Gene in Malaria Patients along Thai-Myanmar and Thai-Malaysian Borders

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ABSTRACT

Introduction: Artemisinin-based combination therapy (ACT) is the first-line drugs for *Plasmodium falciparum* treatment in most countries including Thailand. However, the emergence and widespread of drug resistance malaria throughout the country remain a major problem causing morbidity and mortality.

Objectives: Since *Plasmodium falciparum multidrug resistance 1* gene (*Pfmdr1*) is probably established as a reliable molecular marker for surveillance of the drug efficacy, this study aimed to investigate the polymorphisms of *Pfmdr1* gene in *P. falciparum* malaria in patients who lived along Thai-Myanmar and Thai-Malaysian borders.

Methods: A total of one hundred *P. falciparum* isolates were collected from endemic areas of Thailand along Thai-Myanmar and Thai-Malaysian borders. Five point mutations at codon 86, 184, 1034, 1042 and 1246 in *Pfmdr1* gene were evaluated using polymerase chain reaction restriction fragment length polymorphism (PCR/RFLP). The study protocol has been approved by the Ethics Committee of the Human Ethical Review Board of Thammasat University (no. 112/2562).

Results: Among the enrolled *P. falciparum* isolates, N86Y was the most prevalent *Pfmdr1* gene mutations (53%: Thai-Myanmar 52% and Thai-Malaysian 1%), followed by S1034C (8%: Thai-Myanmar 8%) and N1042D (2%: Thai-Myanmar 2%). All isolates carried wild types at codons 184 and 1246. A significant difference between Thai-Myanmar and Thai-Malaysian was found ($p < 0.05$). Four haplotypes were identified: wild types NYSND (46%), single mutant haplotypes YYSND (47%), double mutant haplotypes YYCND (5%) and triple mutant haplotypes YYCDD (2%).

Conclusions: The prevalence of mutant haplotypes is prominent in Thai-Myanmar isolates compared to Thai-Malaysian isolates. These findings provide evidence of the geographic selection of specific *Pfmdr1* gene mutation haplotypes associated with multidrug resistance in Thailand.

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A015

Using a Small Cancer Drug Library to Study Acquired Vulnerabilities in the Gemcitabine/Cisplatin-Resistant Cholangiocarcinoma

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ABSTRACT

Introduction: Cholangiocarcinoma (CCA) is an aggressive type of cancer commonly found in Thailand. The first-line standard of care treatment is gemcitabine. Many clinical pieces of evidence report a benefit from using a combination of gemcitabine and a platinum agent Cisplatin. Interestingly, GEM/CIS resistance has been found in the patients through the treatment course.

Objectives: To identify potential effective drugs for treating the Gemcitabine/Cisplatin (GEM/CIS) resistant cholangiocarcinoma and molecular pathway(s) underlining the resistance mechanism.

Methods: We assembled a small FDA-approved cancer drug library (62 drugs). GEM/CIS resistant cancer cell lines, TFK1R and KKU213CR were developed. Acquired vulnerability screens were performed using high-content imaging. The results were verified using various pharmacological and cell biology techniques.

Results: We found that LCL161 an IAPs inhibitor showed a potent killing effect on the GEM/CIS resistant cells compared to the parental cells. The results can be consistently observed in both TFK1R and KKU213CR cells. We also confirmed that the combination between GEM/CIS and LCL161 gives a strong synergism and prevents the emerging of the drug-resistant clones in the wild-type cells. Mechanistically, we found that the GEM/CIS resistant cells are prone to apoptosis, because of the reduced level of XIAP and that LCL161 further suppresses the XIAP level toward the caspase 3-dependent programmed cell death.

Conclusions: We discovered an acquired vulnerability of the cancer cells to XIAP inhibition under the GEM/CIS treatment. We also demonstrated that the adaptation opened a new opportunity for the treatment of GEM/CIS resistant CCA by an XIAP inhibitor, which can overcome the GEM/CIS resistance.

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A016

Differentiation Potential of Cholangiocarcinoma Stem Cells Evaluated by Live-biosensor

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ABSTRACT

Introduction: Cholangiocarcinoma, a poor prognosis cancer, has been shown to consist of a high percentage of cancer stem cells, identified by cell surface markers. Here, we constructed a live-biosensor based cholangiocarcinoma stem cell model, which detected the core pluripotency factors (SOX2, OCT4) enabling real-time observation of cancer stem cell plasticity at a single-cell level. Differentiation potential of the cancer stem cells and models had been tested for the model evaluation by two independent methods.

Objectives: The present study constructed a live-biosensor based cholangiocarcinoma stem cell model and evaluated the constructed model by cancer stem cell properties.

Methods: The properties and percentage of cancer stem cells indicated by fluorescent signal were observed by fluorescence microscope and flow cytometry. Differentiation potentials were tested by all-trans-retinoic acid treatment and decaying of the cancer stem cell population in long-term culture.

Results: Using SORE6 biosensor, we had constructed 2 cholangiocarcinoma cell lines showed the different percentage of cancer stem cells in 2D conventional medium. All-trans-retinoic acid treatments were able to show differentiation induction within 3 days in a dose-dependent manner. Differentiation potential as indicated by biosensor positive cells decreased since the early passages of conventional culturing.

Conclusion: Our study, for the first time, demonstrated the live-biosensor as a representative tool to clearly study cholangiocarcinoma stem cells dynamic without relying on cell surface markers. However, cell surface markers of biosensor positive cells need to be validated. We found that cholangiocarcinoma cells contain ~5-15% positive cells depending on culture conditions. In order to study the stringency of the model, we would like to further study this in vitro 3D culture as well as animal model.

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A017

Association between *TPMT* and *NUDT15* Polymorphisms with 6-MP-induced Neutropenia in Thai Pediatric Patients

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ABSTRACT

Introduction: Thiopurine S-methyltransferase (*TPMT*) and nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*) are key enzymes for the metabolism of 6-mercaptopurine (6-MP). In comparison to European populations, the impact of *NUDT15* polymorphism on 6-MP-induced neutropenia is more prominent than *TPMT* polymorphism in Asian population.

Objectives: To elucidate the association between *TPMT* and *NUDT15* polymorphisms and 6-MP-induced neutropenia in Thai pediatric patients.

Methods: One hundred and fourteen Thai pediatric patients treated with 6-MP during 6 months of a maintenance phase for acute lymphoblastic leukemia (ALL) therapy were enrolled. Genotyping for *TPMT* and *NUDT15* was performed by TaqMan SNP genotyping assay and DNA sequencing. The study protocol has been approved by the Ethics Committee of Khon Kaen University.

Results: The risk of 6-MP-induced neutropenia was significantly increased with 7 to 24-fold-higher as early as at the 2nd month and the 4th month in patients who carried *NUDT15**3 and *NUDT15*2* compared with individuals with wild type of both *TPMT* and *NUDT15*. The average absolute neutrophil counts (ANC**)** during 6-month maintenance phase were significantly lower in patients who carried *NUDT15* variants than those with *TPMT* variant.

Conclusions: Genetic polymorphism of enzymes involved in 6-MP metabolism, particularly *NUDT15*, plays a major role in 6-MP-induced neutropenia in Thai pediatric ALL patients. These suggest that the genotyping of *NUDT15* prior to 6-MP prescribing is also necessary to reduce the hematotoxicity of 6-MP in Thai pediatric ALL patients.

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A018

ATIC C347G Polymorphism Influence on Methotrexate Response in Thai Rheumatoid Arthritis Patients

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ABSTRACT

Introduction: Methotrexate (MTX) is the essential disease-modifying anti-rheumatic drug. It is metabolized to methotrexate polyglutamate which inhibits 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (*ATIC*), leading to the release of anti-inflammatory adenosine. Several studies have determined the potential contributions of the *ATIC* polymorphism to the non-responsiveness or toxicity of MTX in rheumatoid arthritis (RA). *ATIC* C347G that related to adenosine formation may contribute to the treatment outcome of MTX in RA patients.

Objectives: To investigate the association of clinical response to methotrexate with *ATIC* C347G polymorphism in Thai RA patients.

Methods: A total of 145 RA patients treated with MTX were included. *ATIC* C347G polymorphism was genotyped, and clinicopathological variables were collected. The genetic polymorphism was analyzed by TaqMan® SNP genotyping assays. The chi-square test was used to assess the association between the good clinical response and nonresponse groups. The clinical response to MTX was analysed using ANOVA. The study protocol has been approved by the Ethics Committee of Khon Kaen University.

Results: The frequencies of *ATIC* C347G (rs2372536) genotypes were 44 CC (30.3%), 66 CG (45.6%) and 35 GG (24.1%). The *ATIC* C347G polymorphism analysis showed a good association with response to MTX for the GG vs. CC genotype (OR 2.962, 95% CI 1.18-7.43, $P = 0.017$). In addition, the association were detected in the recessive model (CG + CC vs GG, OR = 2.538; $p = 0.020$).

Conclusions: Our study suggests that *ATIC* 347GG genotyping would be useful to predict patients who probably get benefit from low-dose MTX treatment in the early phase of treatment.

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A019

Structure-Activity Relationship Study on the Oxyresveratrol Derivatives for COX-2 Inhibitors

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ABSTRACT

Introduction: Oxyresveratrol is a type of natural phenol and a phytoalexin produced in various plants, especially the stem of *Artocarpus lacucha* Buch.-Ham (Mahad). It plays a potent tyrosinase inhibitor, and many biological activities, including possess anti-inflammatory.

Objectives: To isolate and synthesize oxyresveratrol derivatives for evaluation of COX-2 inhibitory activity, MRC-5 cell line viability, and Structure-Activity Relationship study (SARs)

Methods: Oxyreveratrol was extracted from *Artocarpus lacucha* Buch.-Ham with methanol and purified by column chromatography. Its chemical modifications via full-methylation, full-acetylation, and reduction were achieved with excellent yield. Anti-inflammation against COX-2 was measured by enzyme-linked immunosorbent assay (ELISA). Cytotoxicity assay was evaluated by MTT assay in MRC-5 cells.

Results: The heartwood of *A. lacucha* showed a maximum quantity of oxyresveratrol (11.99 % w/w) based on the methanolic crude extract. The presence of reducing oxyresveratrol showed the best result on anti-inflammation (IC_{50} 11.50 ± 1.54 μg/ml) with low cytotoxicity on normal human fibroblast (MRC-5 cells; IC_{50} 106.02 ± 3.86 μg/ml).

Conclusions: The reducing oxyresveratrol product showed the best result on anti-inflammatory with low cytotoxicity on human fibroblast MRC-5 cells.

Acknowledgement: *Drug Discovery and Development center, Office of Advanced Science and Technology*

A020

Effects of *Pluchea indica* Leaves Aqueous Extract on Anxiety, Depression and Memory in Experimental Animals

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ABSTRACT

Introduction: *Pluchea indica* (L.) Less ("Khlu" in Thai) is commercially available in a tea form and traditionally claimed to have beneficial effects in many diseases. The extracts of *Pluchea indica* root and leaves were reported to have high antioxidant activity, increase GABAergic activity in mouse brains and inhibit cholinesterase activity. Thus, *Pluchea indica* may be able to reduce anxiety, depression and improve memory.

Objectives: This study investigated the effects of aqueous extract of *Pluchea indica* leaves on anxiety, depression and memory in experimental animals.

Methods: In male ICR mice, *Pluchea indica* leaves aqueous extract (PIE), at the doses of 50 or 100 mg/kg orally, were tested. The elevated plus maze and dark-light box tests were used as assays for anxiety, forced swim test for depression and Morris water maze test for spatial memory.

Results: A single dose of PIE at 50 or 100 mg/kg showed significant anti-anxiety effect in both elevated plus maze and dark-light box tests. However, only the dose of 50 mg/kg of PIE had a significant anti-depressant effect in the forced swim test. Mice forced fed with either 50 or 100 mg/kg/day of PIE for 10 days showed a significant increase in spatial memory in Morris water maze test.

Conclusions: *Pluchea indica* leaves extract has anti-anxiety and anti-depressive effects. Moreover, the extract also improves spatial memory. The results suggest that *Pluchea indica* leaves may have benefit for brain health.

A021

A Study of Self-Medication Knowledge among Science-program Students at Phramongkutklao College of Medicine Open-House Project

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ABSTRACT

Introduction: Self-medication is the act by which a person, on their own account, chooses to administer medicine to themselves in order to prevent, treat a condition. Science-program students may differ from the general population because they are exposed to knowledge about basic science and medications.

Objectives: This study aimed to determine the self-medication knowledge of the science-program students at Phramongkutklao College of Medicine (PCM) open-house project.

Methods: One thousand science-program students were sampled by simple random sampling from students who visited the pharmacology station during the PCM open-house project and were interested in this study. The questionnaires consisted of 10-true/false questions to explore self-medication knowledge. A descriptive statistic, t-test and 2-way ANOVA were used for data analysis.

Results: A total of 613 subjects completed the questionnaires that shown an approximate response rate of 61.30%. The average score of self-medication knowledge was 5.77±1.56, indicating that students had knowledge scores at a moderate level. Based on the current study, the question that students gave the fewest correct answers was “Anti-inflammatory drugs are medications that destroy or reduce bacterial growth” (19.40%). This study found that gender and education level did not interact with total scores, and the main effects showed that female students had higher scores than male students with the statistical significance ($M=5.81\pm1.55,5.62\pm1.61$; $p<0.05$). Furthermore, students who have domicile in Bangkok had higher scores than students in other parts of the country ($M=6.95\pm0.73,5.77\pm0.32$; $p<0.05$).

Conclusions: This study revealed that self-medication knowledge in science-program students was at the moderate level. They need to be improved. The science program in high school should apply this data for developing courses or activities to promote this knowledge and valid self-medication practices for students.

Acknowledgement: *This work was supported by PCM Academic club and the Department of Pharmacology, Phramongkutklao College of Medicine, Thailand.*

A022

The Novel Mitochondria-targeted Hydrogen Sulfide Delivery Molecules AP39 and AP123 Protect Against UVA-induced Photoaging in Human Dermal Fibroblasts and in Mouse Skin *in vivo*

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ABSTRACT

Introduction: Oxidative stress and mitochondrial dysfunction play a role in the process of skin photoaging via activation of matrix metalloproteases (MMPs) and the subsequent degradation of collagen. The activation of Nrf2, a transcription factor controlling antioxidant and cytoprotective defense systems, might offer a pharmacological approach to prevent skin photoaging.

Objectives: We therefore investigated the protective effect of the novel mitochondria-targeted hydrogen sulfide (H_2S) donors AP39 and AP123, and non-targeted controls on UVA-induced photoaging in normal human dermal fibroblasts (NHDFs) *in vitro* and the skin of BALB/c mice *in vivo*.

Results: In NHDFs AP39 and AP123 (50-200 nM) but not non-targeted controls suppressed UVA (8 J/cm^2)-mediated cytotoxicity and induction of MMP-1 activity, preserved cellular bioenergetics and increased the expression of collagen and nuclear levels of Nrf2. In *in vivo* experiments, topical application of AP39 or AP123 (0.3-1 µM; but not non-targeted controls) to mouse skin prior to UVA (60 J/cm^2) irradiation prevented skin thickening, MMP induction, collagen loss oxidative stress markers (8-OHdG), increased Nrf2-dependent signaling as well as increased mitochondrial MnSOD levels and levels of the mitochondrial biogenesis marker PGC-1α.

Conclusions: Targeting H_2S delivery to mitochondria may represent a novel approach for the prevention and treatment of skin photoaging, as well as being useful tools for determining the role of mitochondrial H_2S in skin disorders and aging.

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PROCEEDINGS

P001

Anti-adipogenic Effect of Standardized Extract of *Centella asiatica* ECa 233 on 3T3-L1 Adipocyte Cells

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ABSTRACT

Introduction: Several human metabolic diseases are correlated with abnormal adipogenesis. *Centella asiatica*, a medicinal herb, has been reported on its hypolipidemic properties.

Objectives: The present study proposed to investigate the effect of standardized extract of *C. asiatica* (ECa 233) on anti-adipogenesis action.

Methods: *In vitro* study assessed ECa 233 on cell viability of 3T3-L1 pre-adipocyte cell line by MTT assay. 3T3-L1 cells were induced to be mature adipocyte and treated with 1, 10 and 100 µg/mL of ECa 233 for 24 and 72 h. The lipid accumulation was examined using Oil red O staining.

Results: ECa 233 did not show any toxicity on pre-adipocyte 3T3-L1. During differentiation process, ECa 233 showed the significant inhibitory adipogenic effect in 3T3-L1 cells following 72 h incubation at concentration of 100 µg/mL.

Conclusions: The study demonstrated that ECa 233 is safe for adipocyte and it possesses anti-adipogenesis by decreased lipid accumulation in 3T3-L1 cells. *C. asiatica* extract may be useful for adipogenic dysregulated diseases. Additional studies might further clarify the detailed mechanism as well as *in vivo* effect of ECa 233.

Keywords: *Centella asiatica*, ECa 233, adipogenesis, 3T3-L1

Introduction

The adipocyte is fat storage cell derived from mesenchymal stem cells which can develop into mature adipocytes by process as known as adipogenesis. Adipogenesis is involved with various transcription factors and specific genes [1, 2] such as CCAAT/enhancer-binding protein (C/EBP)α, C/EBPβ, C/EBPγ and peroxisome proliferator-activated receptor γ (PPARγ) genes. Dysregulation of adipogenesis including adipocyte dysfunction is associated with many human diseases, such as metabolic syndrome [3]. Currently, there are many reports on various natural compounds with anti-adipocyte proliferation property [4].

Centella asiatica (L.) Urban, known as Bau-bog in Thailand, is a plant used in traditional medicine. The active compounds of *C. asiatica* are pentacyclic triterpenoids. The major pentacyclic triterpenoids containing in *C. asiatica* are asiaticoside, madecassoside, asiatic acid and madecassic acids [5]. Additionally, *C. asiatica* contains many phenolic compounds such as flavonoids, catechin, quercetin and glycosides [5, 6]. A standardized extract of *C. asiatica* (ECa 233) is white to off white powder containing triterpenoids of not less than 80% and the ratio between madecassocide and asiaticoside is within 1.5 ± 0.5 [7]. ECa 233 possesses neuroprotective effect [8, 9], anti-inflammatory activity [10-12], wound healing [13] and anti-oxidant effects [14]. To date, no studies have been conducted on anti-adipogenic effect of ECa 233. So that, the present study was proposed to investigate the effect of ECa 233 on adipogenesis of 3T3-L1 pre-adipocytes cell line.

Methods

1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), trypsin/ethylenediaminetetraacetic acid (EDTA), penicillin/streptomycin, L-glutamine and fetal bovine serum (FBS) were obtained from Gibco BRL Life Technologies (NY, USA). Oil red O, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (MO, USA). ECa 233, a standardized *C. asiatica* extract, was provided by Siam Herbal Innovation Co., Ltd (Samutprakan, Thailand).

2. Cell culture and differentiation

Mouse 3T3-L1 pre-adipocytes were cultured in DMEM high glucose supplemented with 10% FBS, 1% penicillin/streptomycin and 1% L-glutamine at 37 °C in 5% CO_2 incubator. Induction of adipocyte differentiation was by incubation of the cells in culture medium

containing 0.25 µM dexamethasone, 0.5 mM 3-isobutryl-1-methylxanthine (IBMX) and 5 µg/mL insulin.

3. Cell viability assay

The cytotoxicity of ECa 233 on 3T3-L1 cells was performed by cultured undifferentiated 3T3-L1 cells in 96-well plate at a density of 1 x 10^4 cells/well overnight. Then, cells were treated with ECa 233 at a concentration of 0, 1, 10, 25, 50, 100 and 250 µg/mL in DMEM high glucose containing 10% FBS for 24, 48 and 72 h. After incubation, undifferentiated 3T3-L1 cells were washed with phosphate buffer saline (PBS) and MTT solution (500 µg/mL) was added for 2 h. The absorbance at 570 nm was read using a microplate reader (BioTek, Highland Park, USA).

4. Oil red O staining

3T3-L1 cells (1 x 10^4 cells/well) were grown on 24-well plate using adipogenic medium. The cells were incubated for 8 days and replaced fresh media every 2 days. During adipogenesis process, cells were treated with 1, 10 and 100 µg/mL of ECa 233 for 24 and 72 h. Thereafter, mature 3T3-L1 adipocytes were washed with PBS and fixed with 4% paraformaldehyde for 40 min. Next, the cells were washed twice in deionized water and incubated in 60% isopropanol for 10 min. Then isopropanol was removed. The cells were washed and incubated with 0.5% Oil red O dye for 40 min at room temperature. Lipid droplets were observed under inverted microscope. For quantitative analysis, 100% isopropanol was used for extracting Oil red O dye from fixed cells and the absorbance was measured at 500 nm.

5. Statistical analysis

An analysis of variance (one-way ANOVA) followed by Least Significant Difference's (LSD's) post hoc test was used for testing the differences among groups. Data was expressed as mean with standard error of the mean (mean ± SEM). Statistical significance was considered when *p* values is less than 0.05.

Results

1. Effect of ECa 233 on viability of 3T3-L1 pre-adipocytes

MTT assay was used to evaluate the effect of ECa 233 on viability of 3T3-L1 pre-adipocyte cell line. The result showed that various concentrations ECa 233 (0, 1, 10, 25, 50, 100 and 250 µg/mL) did not show toxicity effect on cell viability after incubation for 24, 48 and 72 h. Nevertheless, the 250 µg/mL of ECa 233 showed a significant decrease in the viability of cells after 72 h of incubation (Figure 1).

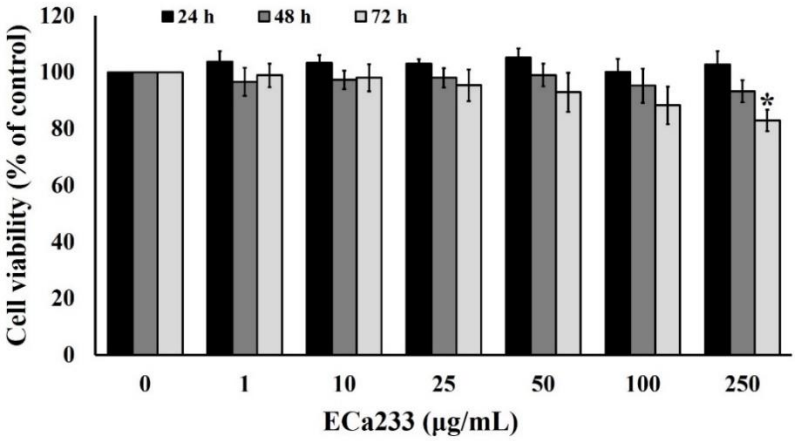

Figure 1. Cytotoxicity of ECa 233 on 3T3-L1 pre-adipocyte cell line. 3T3-L1 cells were treated with various concentrations (1-250 µg/mL) of ECa 233 for 24, 48, and 72 h. Cell viability was determined by MTT assay. Data shown are mean ± SEM of four independent experiments. * $p < 0.05$ compared to the control.

2. Effect of ECa 233 on adipogenesis

To determine the effect of ECa 233 on adipogenesis, pre-adipocyte 3T3-L1 cells were induced to differentiate into mature adipocytes by adipogenic medium for 8 days and followed by treatment with ECa 233 for 24 and 72 h. ECa 233 at the concentration of 100 µg/mL significantly inhibited adipogenesis by decreasing adipocyte number, cell size, and lipid droplet content after 72 h of treatment as shown by Oil red O staining (Figure 2 and Figure 3).

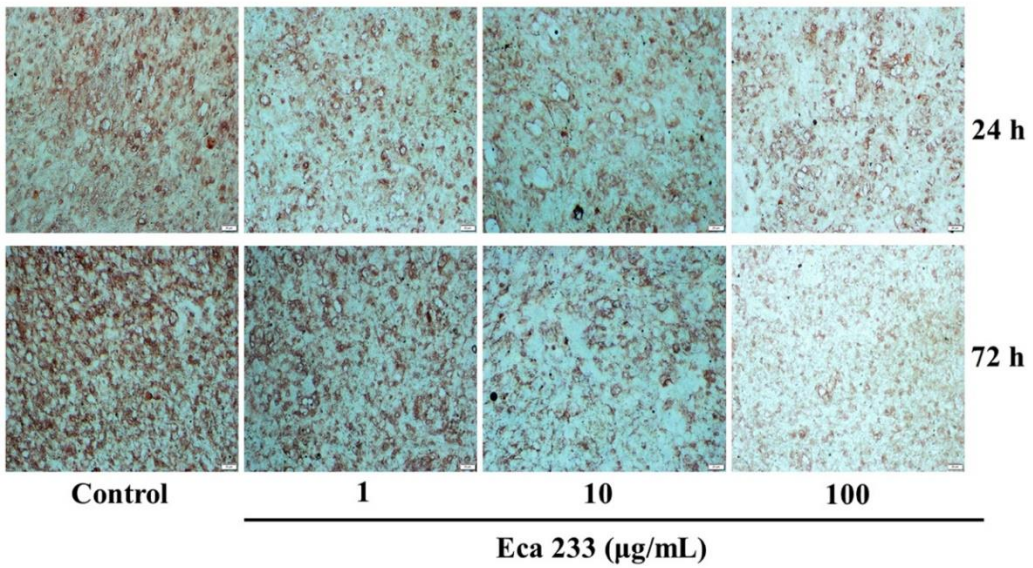

Figure 2. Effect of ECa 233 on adipogenesis in 3T3-L1 cells. The cells were treated with ECa 233 (1, 10 and 100 µg/mL) for 24 and 72 h. The inhibition of adipogenesis was detected by Oil red O staining (20X magnification).

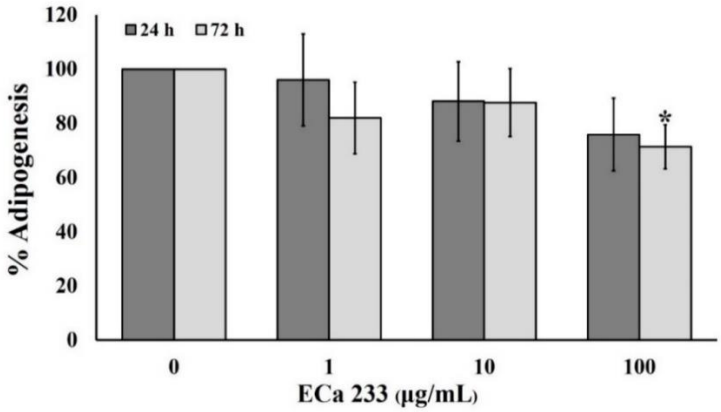

Figure 3. Effect of ECa 233 on adipogenesis in 3T3-L1 cells. Cells were treated with various concentrations (1-100 µg/mL) of ECa 233 for 24 and 72 h. Data shown are mean ± SEM of four independent experiments. * $p < 0.05$ compared to the control.

Discussion and Conclusion

Metabolic dysregulations such as impaired glucose uptake, abnormal insulin secretion, and fatty liver are caused by an improper intake of diets leading to an occurrence of age-associated pathologies like metabolic disorders [3, 15]. Remarkably, the adipocyte dysfunction plays an important role in metabolic disorders such as obesity, liver disorders and type 2 diabetes. Aberrant adipogenesis promotes pro-inflammation and inappropriate release of adipokines, which become health hazard [16]. Therefore, pharmacological intervention that prevents adipocytes differentiation and triggers the uptake of lipid would be a helpful strategy to prevent many diseases. In this present study, it was found that lipid accumulation in the adipocytes was significantly inhibited at the 100 µg/mL of standardized extract of *C. asiatica* (ECa 233) treated group as compared to the control group. Our finding is consistent with the previous study reported that *C. asiatica* extract reduced lipid accumulation during adipocyte differentiation in the coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages [17, 18]. Madecassic acid treatment has improved insulin levels and lowered glucose blood levels by enhanced anti-oxidant and anti-inflammatory effects [19]. Asiatic acid was shown to affect triglycerides level by increased lipoprotein lipase and decreased HMG-CoA reductase activity [20]. In addition, several studies have been reported on various natural compounds such as genistein, anthocyanin, quercetin for their anti-adipocyte proliferation property by suppressing the expression of adipocyte transcription factors, subsequently downregulated lipogenic genes in 3T3-L1 cell [4, 21]. Therefore, ECa 233 inhibits adipogenesis of 3T3-L1 adipocytes which might be due to the substantial components present in ECa 233.

In conclusion, ECa 233 has anti-adipogenic action by reducing lipid accumulation in 3T3-L1 cells. The mechanism of anti-adipogenic effect of ECa 233 should be further elucidated.

Acknowledgement

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Conflict of interest

The authors declare no conflicts of interest.

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P002

Increased Plasma Kynurenine Ratio in Thai Non-small Cell Lung Cancer Patients Correlated with Disease Progression on Immunotherapy

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ABSTRACT

Introduction: Lung cancer is the leading cause of cancer death. Majority of the patient harbored no targetable mutations, and their treatment options are relying on chemotherapy or immunotherapy. Establishment of new predictive biomarkers is important to select the optimal treatment option to maximize the therapeutic benefit. Kynurenine (Kyn), a metabolic product of amino acid tryptophan (Trp) from the enzyme indoleamine 2,3-dioxygenase (IDO), plays important role in immunosuppressive tumor microenvironment and disease progression, and may be a potential biomarker candidate.

Objectives: To evaluate the use of kynurenine to tryptophan (Kyn/Trp) ratio as a biomarker in NSCLC patients without targetable driver mutation, and to determine whether Kyn/Trp ratio correlates with prognosis and response to treatment with durvalumab in stage III NSCLC patients.

Methods: Sixteen NSCLC patients with no actionable mutations who underwent treatment at Ramathibodi Hospital and 17 controls were enrolled. The plasma levels of Kyn and Trp were determined by HPLC and Kyn/Trp ratios were established.

Results: Our results demonstrated the statistically significant increased Kyn/Trp ratios in NSCLC patients compared to the control plasma samples. The Kyn/Trp ratio in control, stage III NSCLC, and stage IV NSCLC groups were (mean ± SD), 0.032 ± 0.007, 0.044 ± 0.014, and 0.085 ± 0.058 respectively. For NSCLC stage III patients who received durvalumab, there were increased in Kyn/Trp ratio in patients with progressive disease (PD). However, due to small sample size, no statistical significance could be concluded.

Conclusions: Kyn/Trp ratios demonstrated an increased trend early on in patients who had progressive disease after treatment with durvalumab and might hold benefit for early detection of disease progression. However, a careful interpretation must be drawn since aging, pregnancy, inflammation, infection and autoimmune diseases could contribute to the increased ratios.

Keywords: NSCLC, kynurenine/tryptophan ratio, biomarker

Introduction

Lung cancer is the most common cause of cancer death worldwide. According to cancer statistics in 2018, the number of deaths from lung cancer is higher than the number of deaths caused by breast, colon, and prostate cancers [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer [2]. Approximately 50% of lung cancer patients have actionable driver mutations such as EGFR, ALK, ROS1, and BRAF that can be treated with targeted therapy with excellent outcome [3]. However, majority of the patients lack an actionable driver mutation and are depended on chemotherapy, and recently, immunotherapy [4]. To date, there is no biomarker to predict response of treatment in this patient population; thus, finding biomarker in order to predict outcome of the disease and response to certain treatments will help maximizing the prognosis.

Immunosuppressive tumor microenvironment (TME) is a major component of the complex tumor architecture. Both cellular and chemical factors work in concert to promote tumor cells proliferation and metastasis [5]. Previous studies showed that kynurenine (Kyn), product of amino acid tryptophan (Trp) from enzyme indoleamine 2,3-dioxygenase (IDO1, IDO2), or tryptophan-2,3-dioxygenase (TDO), is a key factor for immunosuppressive TME [6]. Kyn can induce effector T-cell arrest, promote regulatory T cells (T_{regs}) development, and upregulate PD-L1 expression [7]. Kyn and Trp can be measured in the plasma, and their ratios (Kyn/Trp ratio) are increased in lung cancer patients and associated with disease progression [8].

Approximately 30% of patients with NSCLC is presented at stage III disease. The majority of patients with stage III disease have unresectable tumors. For unresectable patients, standard treatment is definitive chemoradiotherapy. However, median survival is still poor since most patients have disease progression and relapse after chemoradiotherapy. Five-year survival rate for unresectable patients remains at approximately 15% [5]. Until recently, in 2017, durvalumab, a programmed cell death ligand-1 (PD-L1) inhibitor, given after completion of chemoradiation was the first agent that could extend survival in unresectable stage III NSCLC [6].

Since Kyn affects the function of T cells, it could affect the response to durvalumab. Due to high cost of immunotherapy, finding a specific biomarker to select the treatment for each patient is of importance. Thus, this study aimed to identify the role of Kyn/Trp ratios as biomarker in NSCLC patients without targetable driver mutation, and to determine whether Kyn /Trp ratio correlates with prognosis and response to treatment with durvalumab in stage III NSCLC patients. Our long-term goal is to identify the role of plasma Kyn/Try ratio as predictive and prognostic biomarker in NSCLC receiving chemo-immunotherapy.

Methods

1. Sample collection

Plasma samples of non-targetable (without EFGR, ALK, ROS1, and BRAF mutations) stage IV NSCLC patients and stage III NSCLC who have received treatment at Ramathibodi Hospital were obtained (n = 16; stage III n = 11, stage IV n = 5). Control samples were obtained from left-over materials provided by the Department of Pathology (n = 17). Clinical data of the patients and control groups were collected. The study was approved by the Ethics Committee, Faculty of Medicine Ramathibodi Hospital (COA. MURA2020/609), and informed consent was obtained in accordance with the institution guidelines.

2. Detection of plasma kynurenine and tryptophan levels

Two hundred microliters of plasma were mixed with 50 µL of 10% trichloroacetic acid (TCA) for protein precipitation. The acidified plasma was immediately vortexed for 1 min and incubated at room temperature for 15 min before centrifugation at 15,000g for 15 min. The supernatant was filtrated using 0.45 µm nylon syringe filters and fifty microliters of clear filtrate was injected to HPLC system for analysis. Protocol for HPLC detection of plasma kynurenine and tryptophan was applied from Zhen *et.al.* [9]. In brief, BDS-hypersil-C8 column (150 mm x 4.6 mm, 5 µm) was used as stationary phase. The mobile phase consists of 10 mmol/L acetate buffer (pH 4.5) and acetonitrile (94:6, v/v) at a flow rate of 0.8 mL/min. The eluates are monitored by the programmed wavelength UV detection setting at 360 nm for Kyn and at 302 nm for Trp. The plasma samples were measured in triplicated.

3. Statistical Analysis

Data are presented as mean ± S.D. One-way analysis of variance (ANOVA) is adopted, with p value < 0.05 considered as statistical significance. Statistical analyses were performed with GraphPad Prism 9 software (GraphPad, La Jolla, Ca, USA).

Results

Sixteen non-targetable NSCLC patients and 17 controls were recruited to the study. Nine patients (52%) were male and 7 patients (48%) were female. The median age was 64 (40-80) years old for NSCLC and 62 (48-69) years old for control. Seven patients (43.7%) were current smoker, 6 (37.5%) were never-smoker, and 3 (18.7%) were ex-smoker. Eleven patients (68.7%) were at stage III and 5 (31.3%) patients were at stage IV of disease. Majority of the NSCLC patients are adenocarcinoma (*n* = 10, 62.5%) (Table 1).

Table 1. Demographic data

	Patients	Control
Gender		
Male	9	6
Female	7	11
Age		
Mean, range (years)	64 (40 - 80)	62 (48 - 69)
Smoking		
Never smoker	6	
Ex-smoker	3	
Current smoker	7	
Stage		
IIIA	3	
IIIB	8	
IV	5	
Underlying diseases		
None	8	2
DM	2	1
HTN	4	6
DLP	7	10
Histologic subtype		
Adenocarcinoma	10	
Squamous	2	
Unclassified	4	

DM = diabetes mellitus, HTN = hypertension, DLP = dyslipidemia

1. Increased Plasma Kyn/Trp ratio in NSCLC patients

Plasma samples were collected at baseline prior to patient allocation to the appropriate treatment. The ratio between groups were statistically increased in stage III and stage IV NSCLC patients compared to the control group. The Kyn/Trp ratios in control group, stage III NSCLC group, and stage IV NSCLC group were 0.032 ± 0.007, 0.044 ± 0.014, and 0.085 ± 0.058, respectively (Figure 1).

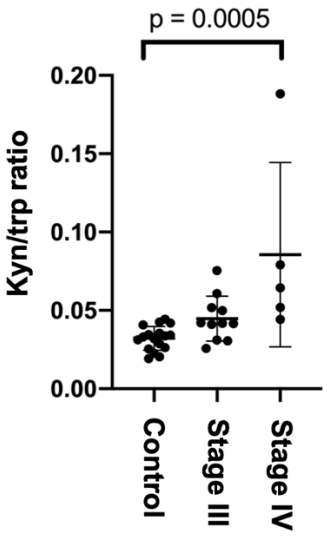

Figure 1. Increased Kyn/Trp ratio in NSCLC patients compared to control.

2. Increased Kyn/Trp ratio in stage III NSCLC patients with progressive disease (PD) after durvalumab treatment

All of the 11 stage III NSCLC patients in the study were given durvalumab at 10 mg/kg every 2 weeks for 12 months or until disease progression. Plasma samples were collected every 2 to 4 months after starting of durvalumab. Two patients had severe hepatitis after first dose of durvalumab and were withdrawn from the study. Two patients had disease progression (PD), as documented by radiographic documentation of distant metastasis (4 and 12 months), and were subsequently removed from the study. Spider plot showed increased Kyn/Trp ratio in NSCLC patients who had PD during durvalumab treatment, while non-PD patients had relatively stable Kyn/Trp ratio below 0.06 (Figure 2).

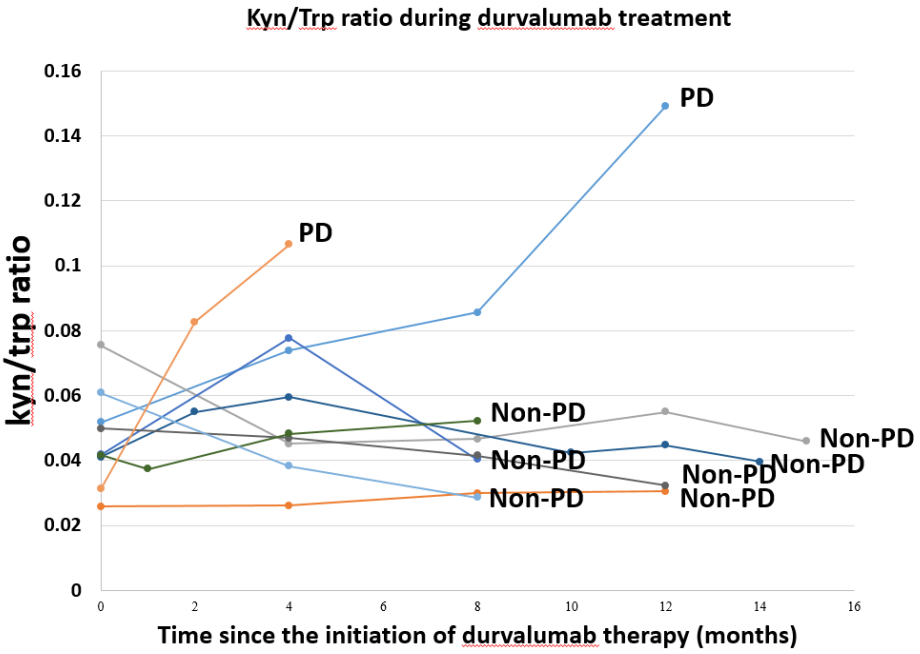

Figure 2. Increased Kyn/Trp ratio in NSCLC patients with progressive disease during durvalumab treatment

3. Stage III NSCLC with progressive disease after durvalumb demonstrated increased trend of Kyn/Trp ratio

The Kyn/trp ratios of lung cancer patients after durvalumab treatment in non-PD group (n = 7) and PD (n = 2) at 4 months were 0.049 ± 0.016 and 0.090 ± 0.023, respectively. The radiological documentation of PD among the two patients were reported at 4 months and 12 months. The Kyn/Trp ratio of PD patients was higher than that of non-PD patients, but it was not statistically significant (p = 0.2545) (Figure 3), due to insufficient number of PD patients to draw a statistically significant conclusion.

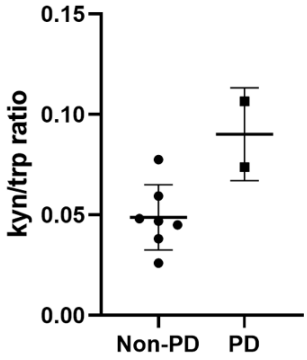

Figure 3. Kyn/Trp ratio between non-PD and PD patients after durvalumab treatment at 4 months

Discussion

NSCLC patients have higher Kyn/Trp ratio than the healthy controls likely due to increased IDO activity, which leads to conversion of tryptophan to kynurenine, resulting in a higher amount of kynurenine [10]. Moreover, this study found that the increased kynurenine is associated with the progressive disease, correlated with the previous data by Suzuki *et. al* [8]. It is possible that kynurenine causes immunosuppression that promotes cancer cells to survive [5]. Despite the increasing trend of Kyn/Trp ratios in PD patients, our baseline plasma Kyn/Trp ratios in non-PD and PD patients did not differ statistically; therefore, Kyn/Trp might not be a good candidate to distinguish patients who are most likely benefit from durvalumab treatment but might rather be an early indicator or progressive disease. There is also an interpretation limitation since kynurenine can be synthesized by various tissues such as liver, and aging, pregnancy, infection, and inflammation could also result in increase in its production. Therefore, larger population size and the use of other metabolites in combination with Kyn/Trp ratio might help better prediction of immunotherapy response. Our small sample size may not be sufficient to draw a statistical conclusion.

Conclusion

In this study, we provided the initial evidence that Kyn/Trp ratio were increased in NSCLC patients and correlated with advanced disease stage. Therefore, it might hold benefit for early detection of disease progression. The future study in larger cohort will be necessary to provide a validation to the findings.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflicts of interest.

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P003

The Differences of TRPA1 Expression Profiles in Dental Pulps to the Inflammation in Deciduous and Permanent Teeth

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ABSTRACT

Introduction: Dental caries can cause inflammation, which involved various mechanisms including stimulation of TRPA1. TRPA1 is one of the non-selective ion channels in human teeth and can expressed due to inflammation from dental caries and acting as a sensor for pain.

Objectives: To determine differences in the distribution of TRPA1 within human deciduous and permanent tooth pulps, both in normal and carious teeth.

Methods: Fifteen permanent and 23 deciduous teeth were obtained from patients requiring dental extractions under local or general anesthesia. After extraction, teeth were prepared and cut into 10 µm sections for indirect immunofluorescence. Double immunofluorescence was applied by using the following combination: (1) β-tubulin III, a general neuronal marker; (2) TRPA1 antigen. Image analysis was then used to determine the mean intensity of immunostaining for TRPA1 by using β-tubulin III as a standard.

Results: Comparing both dentitions, there were TRPA1 expression differences in pulp horn and sub-odontoblastic area in intact dentin group ($p < 0.01$) and also 3 areas in exposed pulp are different in both dentitions ($p < 0.05$)

Conclusions: The differences of TRPA1 expression in deciduous and permanent teeth can imply that mechanisms in responding to inflammation are also different. Even though treatment of dental caries is still similar in both dentitions but with further study it can be reappraised to create more appropriate treatment.

Keywords: TRPA1, dental pulp, dental caries, inflammation

Introduction

Dental caries is an infectious disease composed from various factors including pathogen, sugars, along with host characteristics [1]. Imbalance between risks and protective factors causes tooth demineralization and leads to carious lesion, ranging from subclinical changes to large destruction of tooth structure which can cause undesired inflammation and pain [2]. Sensory nervous system plays an essential part in host defense mechanisms against microbial or foreign-body invasion. They involve in pain reactions as well as nonspecific inflammatory responses. A number of different pain receptor subpopulations exist to be excited by the invasion of noxious stimuli [3]. However, sensory nervous system around orofacial area could differ from other organs. Especially in teeth, nerves are located in the middle of each tooth, embedded in soft tissues called dental pulp, then surrounded by hard tissues, i.e., dentin, andenamel [4] (Figure 1).

Dental pulp is a loose connective tissue, consist of 75% water and 25% organic component. Grossly divided into 2 parts: Coronal pulp (pulp tissues in crown part) and radicular pulp (pulp tissues in the root part). Dental pulp can also be divided by cellular composition into 4 areas; odontoblast area, cell-free zone of Weil, cell-rich zone, and the inner area is the central core [5]. Molecular mechanism of dental pain or sensitivity is still controversial. But some studies showed that many mediators, such as cholinergic, adrenergic and prostaglandin, take role in this mechanism. These mediators associate with many receptors and have various outcomes depend on types of stimuli [6, 7].

However, dental sensory nervous system could also have protective role to help repair hard structure. Inflammation in dental pulp occurred from many pairs of mediators and receptors, and one of the most important receptors is transient receptor potential (TRP) channels. TRP, non-selective ion channels, were first discovered in 1990s in *Drosophila* (fruit fly). In mammals, TRP channels can be found in various organs and related to sensory transduction, mainly from mechanical, chemical, temperature stimuli and other stimuli such as touch, osmolarity, pheromone etc. [8-11].

TRP can be divided into 7 subfamilies; TRPC, TRPV, TRPM, TRPP, TRPML, TRPA, and TRPN [4]. Only 4 subfamilies can be detected in dental tissues including TRPV, TRPM, TRPC, and TRPA. TRPA1(Transient Receptor Potential Ankyrin 1 channel) is the only group in TRPA subfamily presented in many organs such as lungs, pancreas, heart etc., which involved with pain, thermal and chemical stimuli and can be activated by noxious cold and

irritating chemicals such as mustard oil, raw garlic, onion, wasabi, or some specific endogenous compounds [1].

There is still a gap of knowledge about TRPA1 in the fact that TRPA1 can express due to inflammation and pain in dental pulp nerves but histological investigations of caries-induced pulpal changes have been largely limited to permanent dentition, also there is little understanding of how the deciduous tooth pulp responds to this disease.

Therefore, the overall objective of this study was to investigate the presence of TRPs channels within human tooth pulp by using an immunocytochemical approach. The specific objectives were to compare the presence of TRPA1 in intact and carious deciduous and permanent teeth, which can be used to develop drugs or procedures to manage inflammation in teeth or other organs in the future.

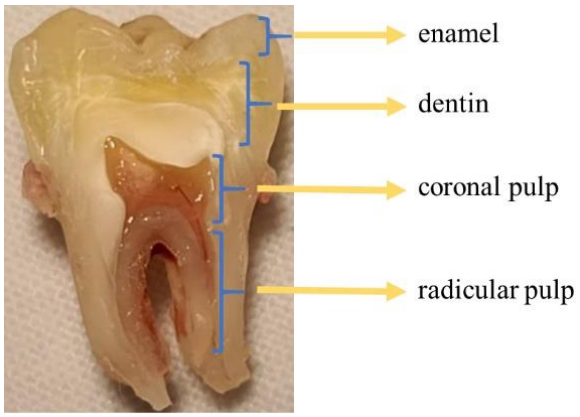

Figure 1. Anatomy of tooth, grossly divided into enamel, dentin, and pulp (coronal and radicular pulp)

Methods

1. Preparation of dental pulp

This experiment consists 38 human teeth (15 permanent teeth and 23 deciduous teeth) extracted under local or general anesthesia, from patients in Songkhla province, Thailand. Deciduous teeth presented with physiologic or pathologic root resorption were excluded.

Patient's information was asked including age and medication taken before the visit. Patients who had taken analgesic drugs within 2 days before the extraction visit were also excluded. The study protocol was approved by the ethics committee of Faculty of Dentistry, Prince of Songkla University (EC6212-057).

After the extraction procedures, the extracted teeth were immediately immersed in a centrifuge tube containing 4% paraformaldehyde (pH 7.4) for 24 h. Teeth were rinsed and

stored in PBS for another 24 h, then immersed in 30% sucrose in PBS for 5 h, and finally were stored in 4 ℃. Afterwards, the pulp in each tooth was removed by making a longitudinal cut with dental high-speed diamond bur and split with a straight dental elevator to separate the buccal and lingual halves. Depth of caries was confirmed with macroscopic eyesight then gently remove the pulp from the half that is more complete in shape.

2. Tissue preparation for immunochemistry

The pulp embedded in OCT compound was longitudinally sliced in to 10 µm thick section by using cryostat set at -20 °C. The sections were collected with silane-coated glass slices and kept in -20°C.

3. Immunohistochemistry

Indirect immunofluorescence was used. Slides were removed from storage and air dry at room temperature. The slides were washed with PBS containing 0.1% triton X-100 (0.1% PBST) (3x10 min), incubated with normal goat serum in 0.25% PBST for 1 h and washed with PBS (3x10 min). Then the slides were applied with primary antibody to TRPA1 (polyclonal rabbit anti-TRPA1, Novus biological) and β-tubulin III (polyclonal mouse anti- β-tubulin III, Abcam) in 5% normal goat serum in 0.1% PBS-T at the dilution of 1:500 and incubated at 4 ℃ for 24 h. Then the slides were washed again with 0.1% PBST (3x10 min) before incubating with secondary antibody; goat anti-rabbit (FITC) and goat anti-mouse (CY3) in 5% normal goat serum in 0.1% PBS-T, at the dilutions of 1:500 and 1:1000, respectively, for 2 h in room temperature, following by applying Vectashield antifade mounting medium and storing at 4℃ until the fluorescence microscopic analysis.

4. Analysis of images

Tissues were examined by using a fluorescence microscope (Zeiss) with analysis software (Zen 2.6 blue edition). Three different areas were studied (Figure 2); pulp horn area, sub-odontoblastic area and mid-coronal pulp area. Mean intensity value was measured in each area from 3 random areas with size of 6400 μm^2 (Figure 3).

5. Statistical analysis

All data were analyzed with SPSS and Microsoft Excel. Independent t-test was used to analyze the mean intensity difference between deciduous and permanent teeth in the same area and depth of caries. The significant levels were set at p value less than 0.05.

Results

Fifteen permanent tooth pulps (6 intact dentin, 6 dentin exposed, 3 pulp exposed) and 23 deciduous tooth pulps (6 intact dentin, 9 dentin exposed, 8 pulp exposed) were stained by indirect immunofluorescence technique to study the difference of the expression of TRPA1 channel between areas in dental pulp and in different depths of caries. As shown in Figure 2, TRPA1 were apparent in green color and it was expressed in all areas observed with different intensity.

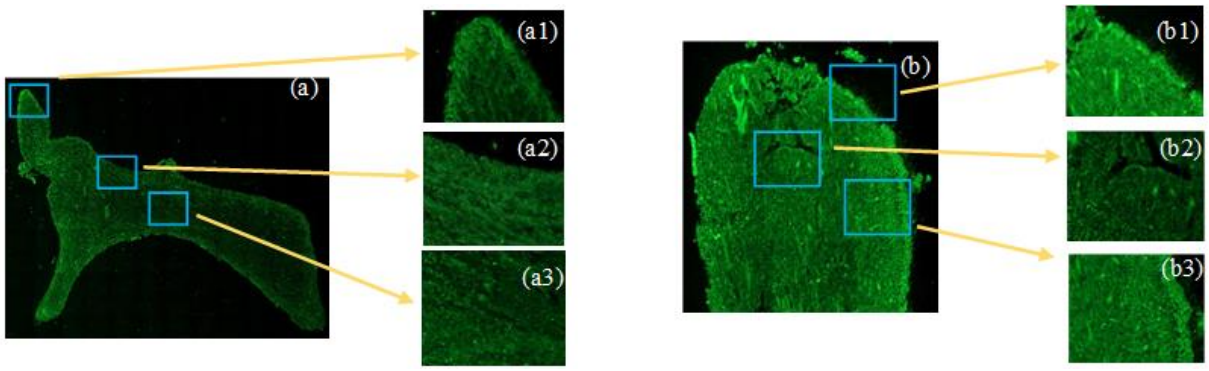

Figure 2 Examples of TRPA1 immunoreactive in (**a**) Permanent teeth (lower left permanent third molar) (**b**) Deciduous teeth (upper right deciduous lateral incisors). Three areas are used in all samples to measure mean intensity of TRPA1: pulp horn area (**a1** and **b1**), sub-odontoblastic area (**a2** and **b2**), mid-coronal pulp area (**a3** and **b3**)

Analysis of TRPA1 expression

After normalizing β-tubulin III, as a marker, the mean intensity of TRPA1 was calculated. The results showed that TRPA1 expression in permanent teeth was most intense at pulp horn area and decreased in sub-odontoblastic area and mid-coronal pulp area, with statistical difference at $p < 0.05$ in intact dentin and dentin exposed (Table 1). But this result was not shown in deciduous teeth pulp. Meanwhile, there was no differences in TRPA1 expression among the depth of caries areas in permanent teeth (Figure 4).

Another interesting result, when compared permanent and deciduous teeth in the same area and depth of caries. Expression of TRPA1 between deciduous and permanent teeth in pulp horn and sub-odontoblastic areas in intact dentin group was statistically different ($p < 0.01$). All areas in dentin exposed group were also statistically different ($p < 0.05$) (Figure 4).

Table 1. Expression of TRPA1 by immunofluorescent staining in permanent and deciduous teeth according to area and depth of caries (mean ± SD).

Area of Interest		Dental caries progression		
		Intact dentin	Dentin exposed	Pulp exposed
Pulp horn	Deciduous	1002.940±151.812	1126.440±225.164	1855.393±445.105
	Permanent	2035.340±485.908	1878.294±650.013	1897.064±694.998
	p value	0.0006**	0.0147*	0.9064
Sub-odontoblastic	Deciduous	908.902±106.871	1059.732±167.940	1718.407±364.633
	Permanent	1283.697±256.585	1304.431±235.855	1511.407±345.840
	p value	0.008**	0.0347*	0.4184
Mid-coronal pulp	Deciduous	876.626±189.904	995.709±120.381	1781.777±368.463
	Permanent	1185.282±282.940	1220.702±233.798	1476.069±564.123
	p value	0.0508	0.0283*	0.3102

* $p < 0.05$
**$p < 0.01$

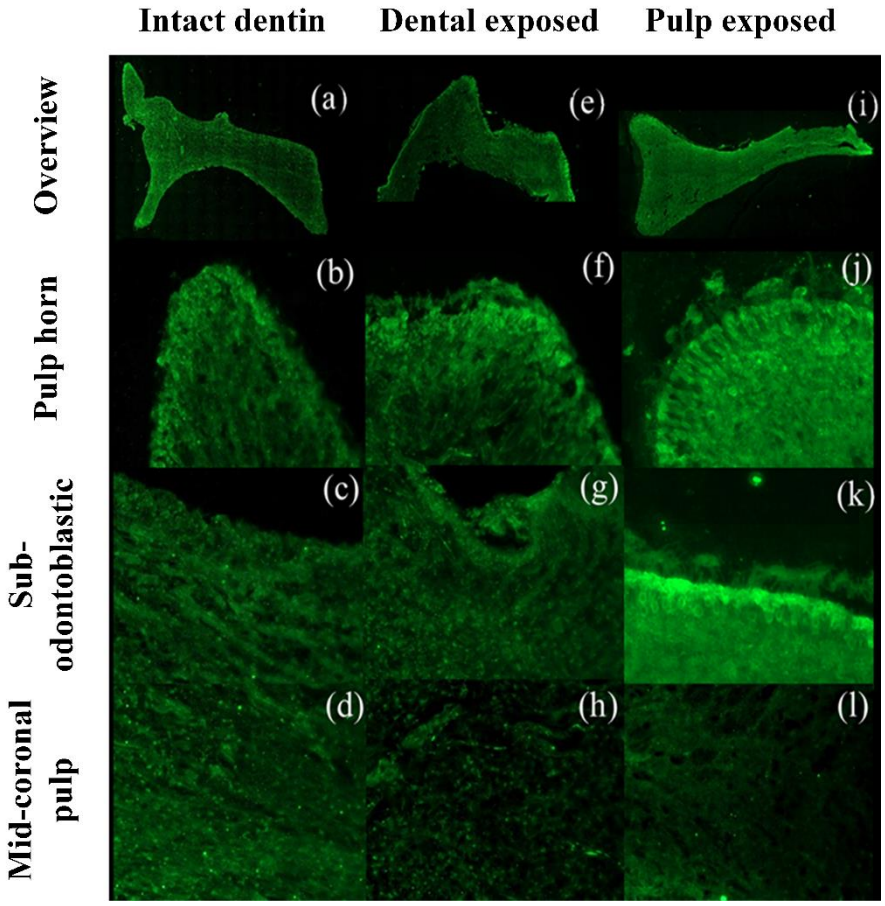

Figure 3A. Comparing TRPA1 immunoreactive in permanent teeth in 3 depth of caries groups; intact dentin (a-d), dentin exposed (e-h), pulp exposed (i-l) and 3 areas of dental pulp; pulp horn area (b, f, and j), sub-odontoblastic area (e, g, and k), mid-coronal pulp area (d, h, and l).

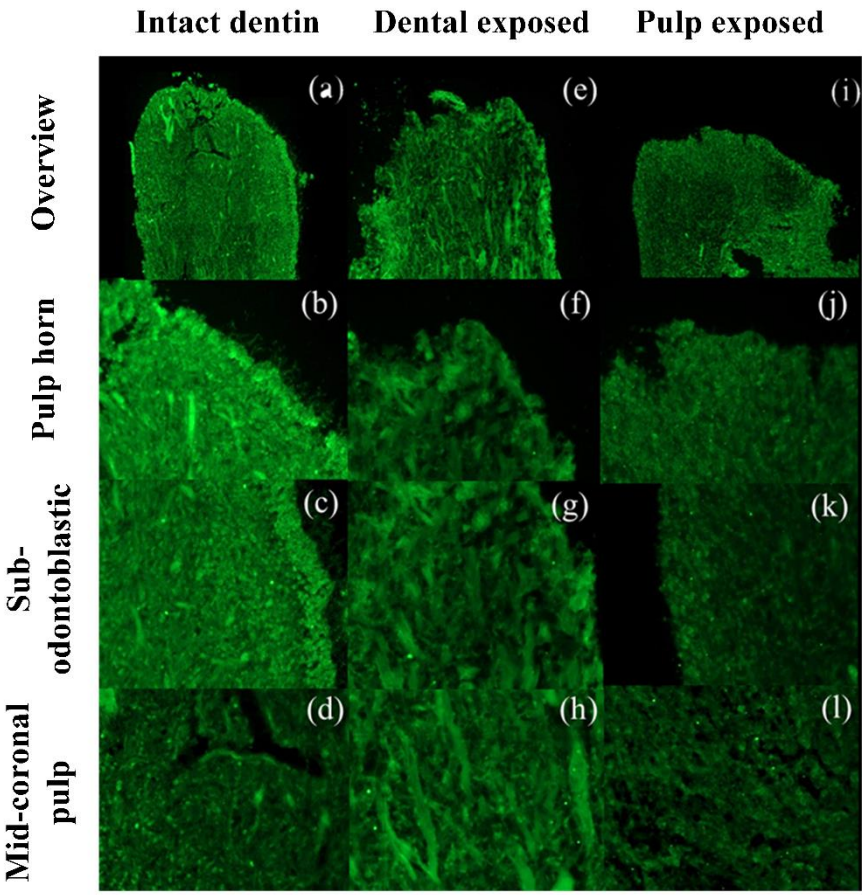

Figure 3B. Comparing TRPA1 immunoreactive in Deciduous teeth in 3 depth of caries groups; intact dentin (a-d), dentin exposed (e-h), pulp exposed (i-l) and 3 areas of dental pulp; pulp horn area (b, f, and j), sub-odontoblastic area(e, g, and k), mid-coronal pulp area (d, h, and l).

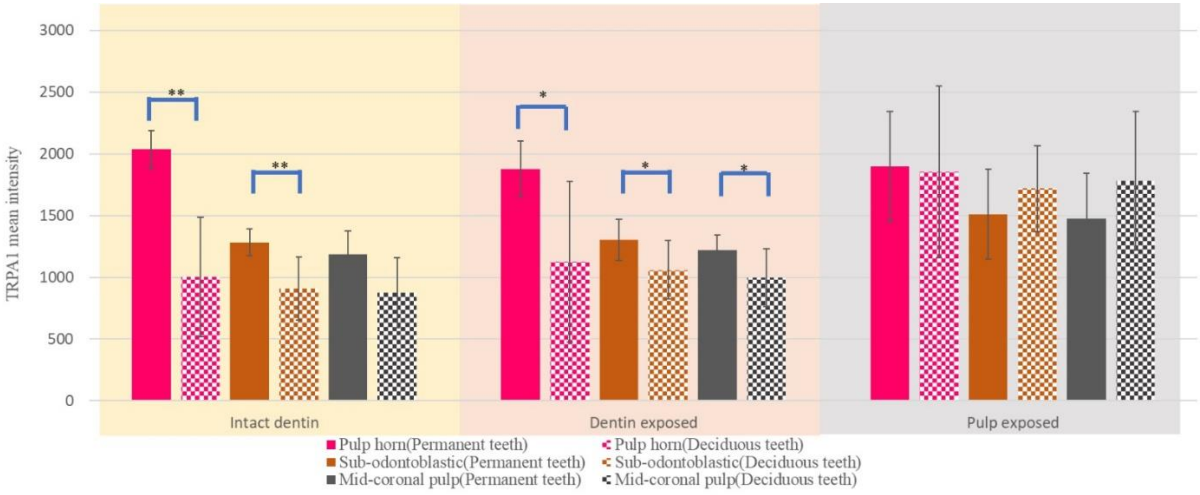

Figure 4. Bar chart indicated mean intensity of TRPA1 (mean ± SD) in permanent and deciduous tooth pulps, comparing in 3 areas and 3 depth of caries groups**.**

Discussion

The presence and distribution TRPA1 are important indicator in the histopathological assessment of pulpal inflammation. Our study attempted to identify the total TRPA1 population within sound and carious tooth pulps by immunofluorescent labelling technique. This technique allows us to visualized total pulpal neuronal profile and review the presence of TRPA1. We focused onto the morphology and distribution of TRPA1 into three regions of dental coronal pulps which were pulp horn, sub-odontoblastic, and mid-coronal pulp area.

Our study shows 2 main findings. First, there was greater number of TRPA1 in permanent teeth than deciduous teeth. Second, there were greater number of TRPA1 presented in pulp horn area than sub-odontoblastic and mid-coronal pulp area, especially in intact dentin and dentin exposed group of permanent tooth pulps, that we found statistically different. Over that past decade, there has been increasing interested in nervous system interaction in relation to inflammation orchestration. Our observation appears to support other neuronal studies.

Consistent with several previous studies [12-14], deciduous teeth pulps were less innervated than permanent teeth pulp, which can explain why deciduous teeth are often less sensitive to stimuli. Also, it was shown that TRPA1 is branched more in peripheral pulp than inner coronal pulp or in radicular pulp [15]. Moreover, TRPA1 is increased by injury or stimuli both in human and animal teeth. The observation made in this study also supports the role of TRPA1 to carious lesion, for not only the defensive role but also the protective role as well [16].

Conclusion

The differences of TRPA1 expression in deciduous and permanent teeth can imply that mechanisms in responding to inflammation are also different between two types of teeth. Even though treatment of dental caries is still similar in both dentitions, further study may shade some more understanding in designing more appropriate treatment.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflicts of interest.

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P004

Anticancer Activity of *Spilanthes acmella* Murr Extract on Cholangiocarcinoma Cells

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ABSTRACT

Introduction: Serious side effects of chemotherapy and chemoresistance are limitations of cholangiocarcinoma (CCA) treatment. Currently, anti-cancer effects of herbs are used in relief and treatment or used as an alternative medicine. *Spilanthes acmella* Murr. (SA) extract consists of various bioactive compounds and may have anticancer effect.

Objectives: To investigate effects of SA extract on anticancer activity against CCA.

Methods: KKU-100 cells were used to study the effects of SA extract on cytotoxicity, apoptosis and cell death, colony formation, migration, and intracellular reactive oxygen species (ROS) generation. Cytotoxicity was measured by MTT assay. Apoptosis and cell death was assessed by flow cytometric method. Cell migration was performed by wound healing assay. ROS was detected using DCFH-DA fluorogenic dye staining.

Results: SA extract could inhibit cell proliferation with IC_{50} values of 59.6 and 13.8 µg/mL at 24 and 48 h, respectively. SA extract significantly induced apoptosis and cell death, inhibited colony formation in a dose-dependent manner, and suppressed migration at 48 h. SA extract induced generation of intracellular ROS.

Conclusions: SA extract is a plant with the potential for developing chemotherapies for CCA patients.

Keywords: *Spilanthes acmella* Murr., anticancer, cholangiocarcinoma

Introduction

Cholangiocarcinoma (CCA) is a malignant cancer of the bile duct and classified into intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA). The highest occurrence of patients with CCA was found in the Northeast of Thailand [1, 2]. Its major risk factor is *Opisthorchis viverrini* liver fluke infection [3]. The prognosis of the disease is poor, because of the late diagnosis and poor response to chemotherapy. However, serious drug side effects and chemoresistance are major problems of the treatment. Therefore, a novel drug treatment with high efficacy in anticancer properties is greatly desired.

One of Thai traditional medicine plants is Phak-Kratt-Huawaen. The science name is *Spilanthes acmella* Murr. (SA), a member of Asteraceae family. This plant has been used as a traditional herb to treat toothache, fever, headache, asthma, sore throat, haemorrhoids and rheumatism [4]. The bioactive compounds of SA have been studied which include flavonoids, coumarins, alkamides, sterols, essential oil, fatty acid, phenolic and terpenes [5-8]. Pharmacological properties include antipyretics, antioxidant, anti-inflammatory, antimicrobial, antifungal, vasorelaxant activity, immune-stimulant and anticancer [6]. A previous study has found that SA extract has an anticancer activity against Chinese hamster carcinoma cell, human epidermal carcinoma, human breast adenocarcinoma, human small cell lung carcinoma and human cervical carcinoma cells [9]. However, studies of anticancer properties of SA are rare. There has not been any research on anticancer activity in CCA cells, and the mechanism of action against cancer is unclear.

The present study aimed to investigate the effects of SA extract on CCA cells with a view of assessing any anticancer activity. Cytotoxicity, apoptosis and cell death, colony formation, migration and intracellular ROS generation were determined.

Methods

1. Reagents

Cell culture medium Ham-12, fetal bovine serum (FBS), dimethylsulfoxide (DMSO), and streptomycin-penicillin reagents were purchased from Gibco BRL Life Technologies (Grand Island, NY, USA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma-Aldrich Inc. (Saint Louis, MO, USA).

2. Cell cultures

Human KKU-100 CCA cells were used in this study. KKU-100 cells were kindly provided from Prof. Dr. Veerapol Kukongviriyapan, Department of Pharmacology, Faculty of Medicine and the Cholangiocarcinoma Research Institute, Khon Kaen University, Thailand. The cells were cultured in Ham's F12 medium with 12.5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.3, 100 U/mL penicillin, 100 µg/mL streptomycin and 10% fetal bovine serum (FBS). The cells were maintained at 37°C with 5% CO_2 and sub-cultured every 3 days using 0.25% trypsin-EDTA as a method previously described [10].

3. Preparation of *Spilanthes acmella* extraction

The extract of *Spilanthes acmella* (SA) was kindly provided from Prof. Dr. Supaluk Prachayasittikul**,** Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand. The method of plant collection and extraction were described in a previous study [4]. Aerial parts of plant were stored in the Forest Herbarium, Royal Forestry Department, Nakornsrithammarat Province, Thailand with voucher number BKF 112361. Plant extract was prepared by the Department of Chemistry, Faculty of Science, Srinakharinwirot University. Briefly, air dried SA (1,050 g) was ground and extracted with ethyl acetate (3×5 days), followed by filtration. The filtrates were combined and evaporated *in vacuo* to give a crude ethyl acetate extract (18 g). *Spilanthes acmella* in ethyl acetate extract was dissolved in DMSO for use in experiments.

4. Assay of cytotoxicity

KKU-100 cells were seeded onto 96-well plates at a density of 7.5×10^3 cells/well. SA extract was dissolved in DMSO and diluted with medium to various concentrations and incubated for 24 and 48 h. The cytotoxicity was assessed by the MTT assay. Cytotoxicity was calculated as the percent absorbance of controls. The IC_{50} value was calculated by a nonlinear curve-fitting program.

5. Flow cytometry

KKU-100 cells were treated with 3.125, 6.25, 12.5, 25, 50, 100 µg/mL of SA extract for 24 and 48 h. Annexin V and 7-AAD kit (Merk Millipore, Germany) was used to detect apoptosis profiles (early and late apoptosis). Total cell population was analyzed by flow cytometry (Guava EasyCyte HT, Millipore, Bedford, MA, USA).

6. Colony formation assay

KKU-100 cells were seeded at a density of 600 cells/well onto a six-well culture plate. Cells were treated with 6.25, 12.5, 25, 50, 100 µg/mL of SA extract for 24 h and the medium was changed and incubated for another 8 days with renewal with fresh medium every 3 days. Crystal violet was used for colony staining. The number of colonies were counted under a microscope.

7. Wound healing assay

KKU-100 cells were seeded onto a 24-well plate at density of 2.5×10^5 cells/well. The sterile 200 µL pipette tip was used for a scratch wound. Detached cells were washed with PBS. Cells were treated with 6.25, 12.5, 25, 50 µg/mL of SA extract. Images of scratch wounds were taken at 0, 24 and 48 h. Wound width was measured by Image J software. The migration index was calculated by the ratio of net wound width at the given time and the width at the initial time.

8. Reactive oxygen species assay

KKU-100 cells were seeded onto an 8 well-chamber at density 25×10^3 cells/well and allowed to grow and adhere overnight. Intracellular ROS was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Invitrogen, CA, USA), a fluorogenic dye, according to the manufacturer's instructions. The cells were incubated with SA extract for 24 h and stained with DCFH-DA for 1 h. The fluorescence intensity was measured using fluorescence microscopy with excitation and emission of 485 and 528 nm, respectively.

9. Statistical analysis

The data was analyzed using Student's t-test and one-way analysis of variance (ANOVA) with significant differences between control at P-values < 0.05. All results were presented as the mean ± SEM.

Results

1. Effect of SA extract on cytotoxicity

To assess whether the SA extract inhibited KKU-100 cells growth, cytotoxicity was measured using an MTT assay. Results indicated that SA extract induced cytotoxicity in a time-dependent manner, as shown in Figure 1; IC_{50} values of the extract in KKU-100 cells were calculated as 59.6 and 13.8 µg/mL for 24 and 48 h incubation, respectively (Table 1). The estimated maximal cancer cell killing effect was 100%.

Table 1 IC_{50} values of SA extract on KKU-100 cells.

***Spilanthes acmella* extract**	**KKU-100**	
	24 h	**48 h**
IC_{50} (µg/mL)	59.6	13.8

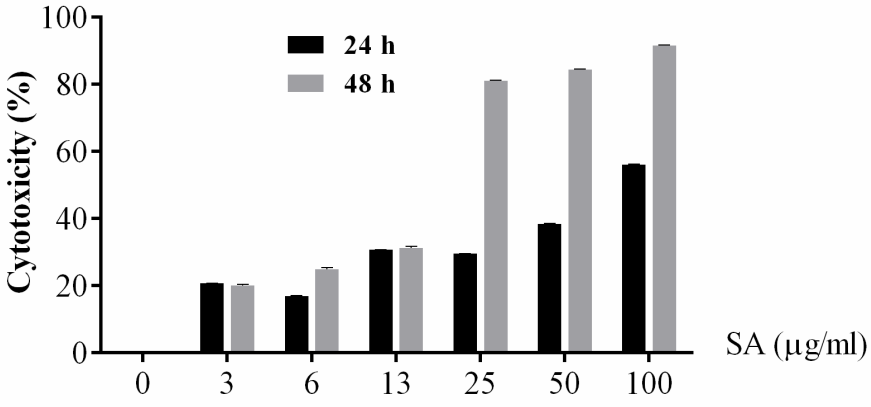

Figure 1. Cytotoxicity of SA extract against KKU-100 cells

2. SA extract induced apoptosis and cell death

KKU-100 cells were treated with SA extract at various concentrations for 24 and 48 h. Cells were stained with annexin V and 7-AAD dye and evaluated apoptosis using flow cytometry. Results revealed that SA extract could induce apoptosis and cell death with the high concentration at 24 h. For 48 h incubation, the low concentration of SA extract also significantly induced apoptosis and cell death (Figure 2A-C).

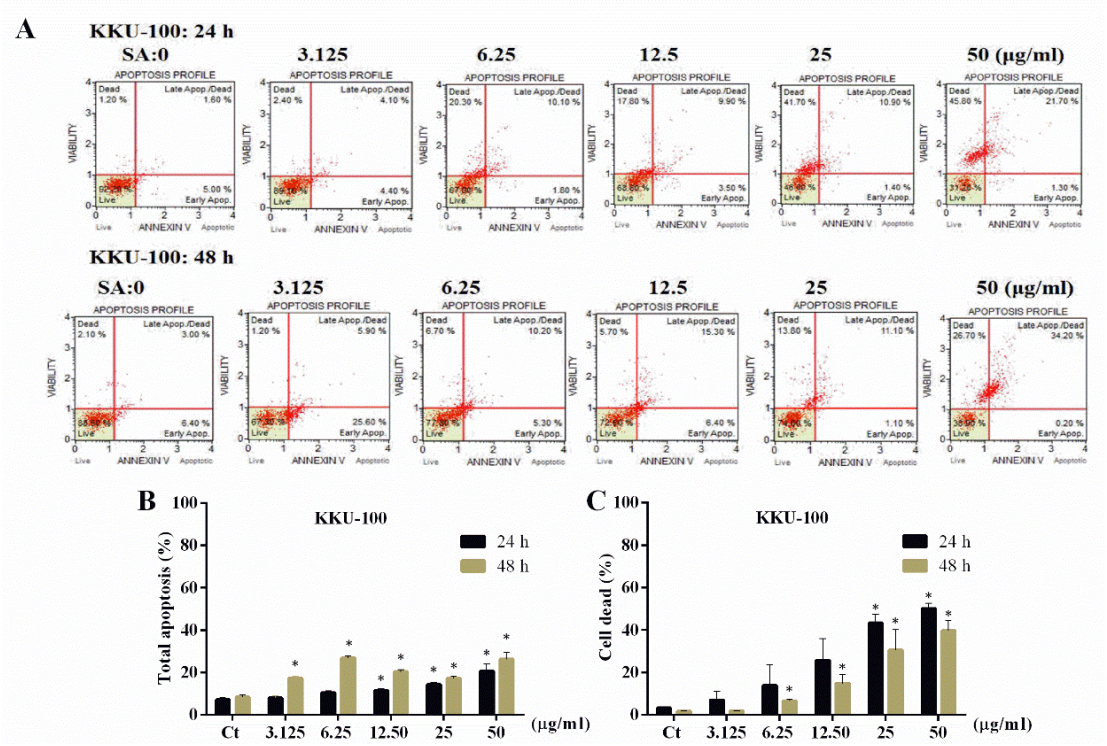

Figure 2. SA extract induced apoptosis and cell death. KKU-100 was treated with SA extract at various concentrations for 24 and 48 h. Cells were stained with annexin V and 7-AAD dye and subjected to analysis using flow cytometry. **A**. Plots of flow cytometry were indicated live, early apoptosis, late apoptosis and dead cell from one experiment. **B**. The numbers of total apoptosis are shown as a percentage. **C**. The numbers of dead cell are shown as a percentage. Each bar represents the mean ± SEM from three independent experiments. *$p < 0.05$ vs. control group. SA; *Spilanthes acmella* extract.

3. SA extract suppressed colony formation

KKU-100 cells were treated with SA extract for 24 h and the colony formation was evaluated. SA extract significantly suppressed the colony formation in a concentration-dependent manner (Figure 3A-B). The percentage of number of colonies when cells were treated with 6.25, 12.5, 25, 50, and 100 µg/mL of SA extract was 96.5 ± 6.4, 80.7 ± 7.6, 70.0 ± 9.3, 66.5 ± 9.8 and 26.8 ± 6.7%, respectively.

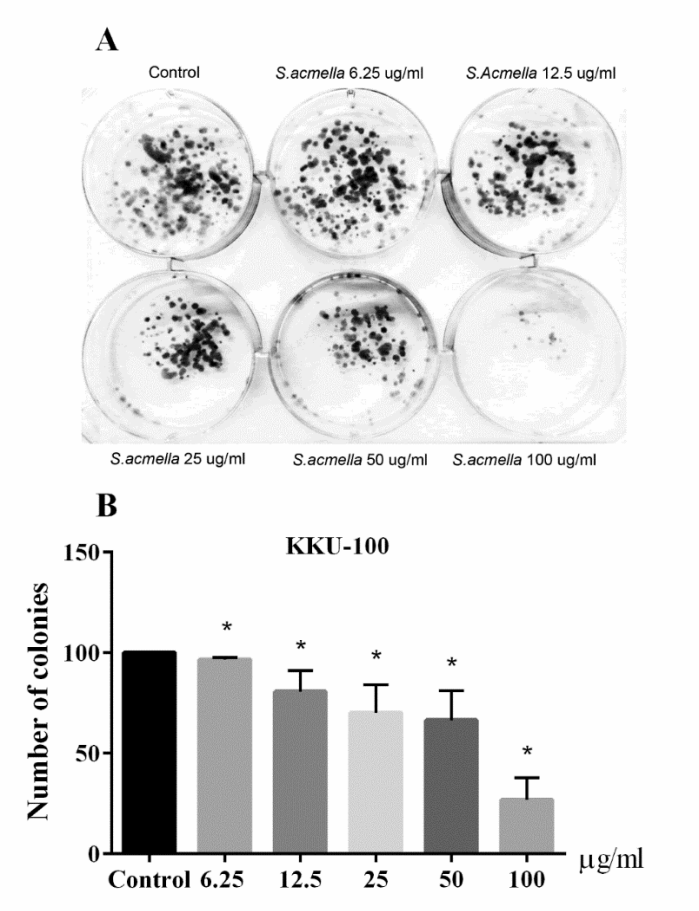

Figure 3. The effect of SA extract on colony formation. KKU-100 cells were treated with SA extract at various concentrations for 24 h. **A**. A series of images are presented for colony formation from one experiment. **B**. The number of colonies are presented as percentages. Each bar represents the mean ± SEM from three independent experiments. *$p < 0.05$ vs. control group.

4. **SA extract suppressed migration**

Cancer migration is one of cancer's hallmarks for metastasis and survival. To investigate the effect of SA extract on migration, wound healing assays were performed after KKU-100 cells were treated with SA extract for 24 and 48 h. Results indicated that the migration index was significantly decreased by 60, 84 and 90% when cells were treated with 12.5, 25 and 50 µg/mL of SA extract, respectively at 48 h. After 24 h, SA extract treated cells could not suppress migration in KKU-100.

5. SA extract stimulated ROS

KKU-100 cells were treated with SA extract for 24 h and intracellular ROS was measured. The data indicated that SA extract stimulated intracellular ROS generation in a dose-dependent manner when compared with the control group (Figure 5).

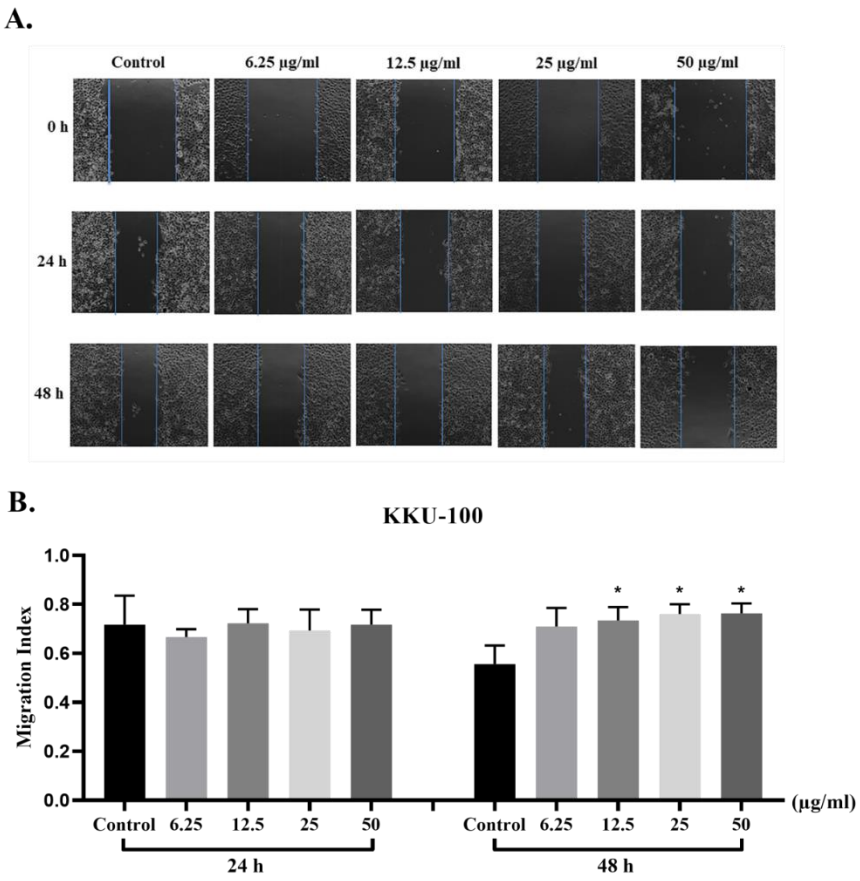

Figure 4. The effect of SA extract on migration. KKU-100 cells were treated with SA extract at various concentrations for 24 h and 48 h. **A**. A series of images are presented cells migration from one experiment. **B**. The number of colonies are presented as percentages. Each bar indicates the migration index and represents the mean ± SEM from three independent experiments. *$p < 0.05$ vs control group.

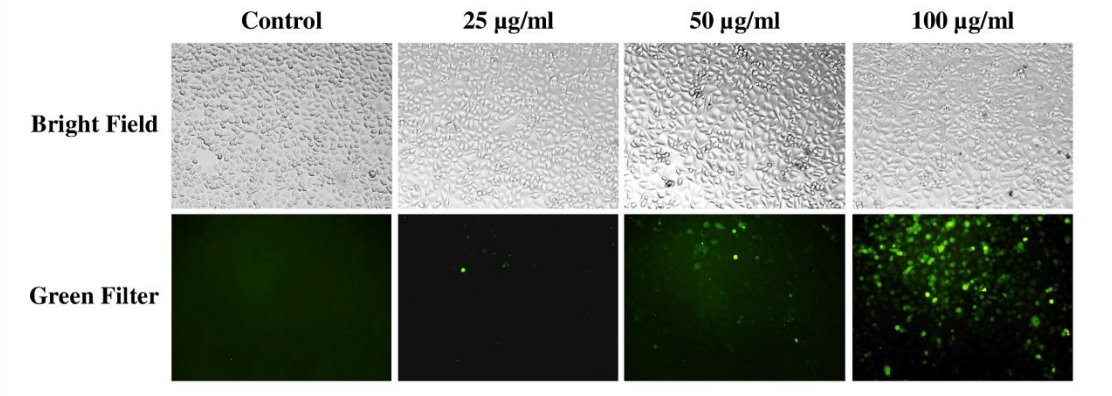

Figure 5. The effect of SA extract on ROS generation. KKU-100 cells were treated with SA extract at various concentrations. After 24 h, cells were stained with DCFH-DA. The intracellular ROS level was determined by fluorescence microscopy. Green color intensity is representative of intracellular ROS. A series of images are indicated from one experiment.

Discussion

Current chemotherapeutic agents still cause serious side effects and chemoresistance leads to less effective cancer treatments and poor quality of life. Many mechanisms of chemoresistance have been proposed, such as apoptosis, up-regulation of antioxidant genes and the EGFR growth pathway [11-13]. Development of natural products is the strategy to improve the effectiveness of chemotherapeutic agents and overcome cancer chemoresistance. SA is a medical plant has been used to relieve pain, and for anti-inflammation and anti-infection, with various bioactive compounds and pharmacological effects (6). Anti-cancer effects of SA have been shown in lymphoma cells and breast cancer [14, 15]. There are no reports concerning anticancer activities of SA in CCA.

Our study is the first to show that SA extract affects CCA cells. SA extract induced cytotoxicity in a time-dependent manner by apoptosis and cell death stimulation in KKU-100 cells with IC_{50} values of 59.6 and 13.8 µg/mL for 24 and 48 h, respectively. A low dose of SA extract was effective in suppressing replicative ability in a dose-dependent manner. Moreover, 12.5, 25 and 50 µg/mL of SA extract significantly suppressed migration in KKU-100 cells. Previous studies have shown that SA extract was effective on MCF-7 breast cancer cells by increasing the cell death, decreasing colony forming ability and suppressing metastasis in a dose- and time-dependent manner [15]. SA exhibited the properties of anticancer through many bioactive compounds, such as flavonoids and sesquiterpene lactones, which have an antiproliferative activity on cell lines, including skin epidermoid carcinoma, breast epithelial adenocarcinoma, and cervix epithelial adenocarcinoma [16]. Moreover, scopoletin and vanillic acid are also bioactive components of SA extract which have been reported to kill cancer cells [17, 18]. The major bioactive compound of SA is an alkylamide-like spilanthol, which has shown analgesic activity [19], local anesthetic activity [20], and antiinflammation activity by inhibition of the NF-κB signaling pathway, suppression of COX-2 and iNOS, and induction of free radical scavenging activity [21]. However, effect of spilanthol on anticancer has not been reported.

Notably, SA extract induced intracellular ROS stimulating in a dose-dependent manner. ROS is a product from intracellular metabolism and important role in cell apoptosis, autophagy, proliferation and differentiation [22]. ROS can cause excessive DNA damage and activate an apoptosis pathway [23, 24]. The compound that induced ROS generation has been proposed for anticancer and enhancing the chemosensitizing effects [25]. The paradoxical finding to the previous report indicated that SA extract has the potential to prevent DNA damage by free

radical scavenging in Chinese hamster carcinoma cell and Dalton's lymphoma ascites, but only had anticancer effects on lymphoma [14]. Therefore, SA extract shows a promising potential anticancer effect on CCA.

Conclusion

Our results suggest that SA extract could suppress the viability, colony formation abilities and migration, and activated apoptosis of KKU-100 cells via regulating intracellular ROS. However, the detailed mechanism of action will need further investigation.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflicts of interest.

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P005

TRPA1 and TRPV1 Profiles in Deciduous Sound and Carious Pulps

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ABSTRACT

Introduction: Inflammation of tooth pulp occurred from various factors such as bacteria invasion due to dental caries. The transient receptor potential ankyrin 1 (TRPA1) and the transient receptor potential vanilloid 1 (TRPV1) are nonselective ion channel receptors contributed in inflammatory process of dental pulp.

Objectives: We aimed to depict the overall profile of TRPA1 and TRPV1 at different stages of carious progression.

Methods: 23 primary teeth were obtained from children requiring dental extractions under local anesthesia or general anesthesia. Following exodontia, 10 µm-thick frozen pulp sections were processed for indirect immunofluorescence. Triple-labelling regimes were employed using combinations of the following: (1) β-tubulin III, a general neuronal marker (2) TRPA1 antigen; and (3) TRPV1 antigen. Image analysis was used to determine the intensity of immunostaining for TRPA1 and TRPV1 by using β-tubulin III as a standard.

Results: Analysis of intensity of TRPA1 and TRPV1 at 3 areas (pulp horn, subodontoblast and midcoronal) of 3 caries groups including intact dentin, dentin exposed and pulpal exposed, revealed that the pulpal exposed group expressed TRPA1 significantly greater than dentin exposed and intact dentin in all areas ($p < 0.01$). In contrast, the intensity of TRPV1 in three groups was significantly changed only at midcoronal area.

Conclusions: TRPA1 was up-regulated according to degree of caries progression but not found this relation in TRPV1.

Keywords: dental caries, carious progression, TRPA1, TRPV1

Introduction

Nature of chronic inflammation has been widely studied. It has been described into the degree of clinical symptoms. The histology of chronic inflammation has previously been shown to be complicated, since it involves many signaling molecules. Therefore, the molecular characteristics of chronic inflammation could not yet be fully described.

The mechanisms of chronic inflammation could be induced by various factors such as infectious agents, bacterial components, foreign bodies, products of metabolisms, immune reaction, or even unknown causes [1-3].

Dental structure is composed of hard and soft structures which can be defined as a unique organ. Since, there are both organic and inorganic structures within the dental pulp. The forming of 30% organic structure in aqueous solution are surrounding by 70% inorganic hard structure to form dentine-pulp complex. Moreover, the odontoblast that is a special cell found in dentine of tooth organ can produce and repair inorganic structure of dentine called reparative dentine [1, 4, 5].

The organic structure in dental pulp does not only provides nutritional function to support dental structure, but also has its own sensory function to induce pain which is an important protective role. Biochemical properties of pulp are unique because of the “gel-like properties” are consisted of protein-rich in water content called “matrix” to influence cells’ behaviors [5].

Cellular properties of dental pulp of outer surface to inner pulp can be divided into several zones; odontoblasts are aligned around cell-free zone and cell-rich zone. While the cell-rich zone and central core of dental pulp are composed of various types of connective tissues such as fibroblasts and mesenchymal cells including capillary and nerve fibers innervated from mandibular and maxillary branches of cranial nerve V (CN V2, V3) [5].

For orofacial region, sensory nervous system is innervated with Aδ and c fibers from maxillary and mandibular branches of CN V [5-7]. There have been widely shown numerous ion channels involved in the transduction process to stimulate nerve impulses such as transient receptor potential channels (TRPs) [3].

TRPs are nonselective ion channels and can be divided into 7 subfamilies. Only 4 subfamilies can be detected in dental tissues including TRPV, TRPM, TRPC, TRPA. TRPA1 and TRPV1 has been detected in dental primary afferent neurons and odontoblasts which involve in dental pain pathway. Both channels can be activated by thermal, chemical stimuli or

some inflammatory mediators indirectly [6, 8, 9]. Additionally, TRPA1 is co-expressed with TRPV1 [10-12] and have the interaction between the two channels [10-14].

Recent studies have described the distinct characteristics of TRPs channels during bacteria invasion due to dental caries regression largely limited to the permanent dentition. However, the responds of primary tooth pulp still have little understanding. In this study, we aim to explore the dynamic of neuronal profiles of TRPA1 and TRPV1 during bacterial infection into the dental pulp to help better understanding of neuronal responses of the defensive mechanism of dental structure in primary tooth.

Methods

1. Preparation of dental pulp

The experimental materials were 23 primary teeth which were obtained from children in Songkhla province, requiring dental extractions under local or general anesthesia. The study protocol has been approved by the ethics committee of Faculty of Dentistry, Prince of Songkla University, and informed consent was obtained from accompanying guardians to use of the extracted teeth for research purposes.

The teeth presented with physiologic or pathologic root resorption were excluded from the study. Patient's informations were collected including age and medication taken before the visit. Patients who had taken analgesic or antibiotic drugs were also excluded.

After obtaining permission, the extraction was performed and the extracted teeth were immediately fixed with 4% paraformaldehyde (pH 7.4) for 24 h. Then the teeth were rinsed and stored in 0.1 M phosphate-buffered saline (PBS) for 24 h, and then in 30% sucrose in PBS for 5 h. Throughout this procedure the teeth were completed soaked in the mentioned liquid and restored in 4 °C.

Afterwards, the pulp in each tooth was removed by making a longitudinal cut with dental high-speed diamond bur and split with a straight dental elevator to separate the buccal and lingual halves. Depth of caries was confirmed with macroscopic eyesight then gently remove the pulp from the half that is more complete in shape.

2. Tissue preparation for immunochemistry

The pulp was embedded in OCT compound and longitudinally sliced into 10 µm thick section by using cryostat set at -20 °C. The sections were collected with silane-coated glass slices and kept in -20 degree Celsius.

3. Immunohistochemistry

Indirect immunofluorescence was used. Slides were removed from a storage and air-dried at room temperature. The slides were washed with PBS with 0.1% triton X-100 (0.1% PBST) (3x10 minutes), then incubated with normal goat serum in 0.25% PBST for 1 h, washed with PBS (3x10 minutes). Then slides were applied with primary antibody to TRPA1, TRPV1 and β-tubulin III (polyclonal rabbit anti-TRPA1(1:500; Novus biological), polyclonal guinea pig anti-TRPV1(1:500; Thermo Fisher) and polyclonal mouse anti-β-tubulin III (1:500; Abcam) diluted in 5% normal goat serum in 0.1% PBST). The slides were incubated at 4 °C for 24 h, then washed with 0.1% PBST (3x10 minutes) and applied with secondary antibody; goat anti-rabbit (FITC, 1:500), goat anti guinea pig (Dylight 350, 1:500) and goat anti-mouse (CY3, 1:1000) diluted in 5% normal goat serum in 0.1% PBST, incubated for 2 h in room temperature, washed and analyzed under fluorescence microscope.

Tissues were examined by using a Zeiss fluorescence microscope with analysis Zen software (Zen 2.6 blue edition). 3 different areas were studied; pulp horn, subodontoblastic and midcoronal. 20x objective was used to investigate. Computer-assisted image analysis software (Zen software) was used for automatic calculation of the mean intensity represented 6400 μm^2.

4. Statistical analysis

Data were analyzed by SPSS Statistics and Microsoft Excel. Difference of the mean intensity of TRPA1, TRPV1 in each area (pulp horn, subodontoblastic, midcoronal) and each degree of caries progression (intact dentin, dentin exposed, pulpal exposed) was analyzed by one-way ANOVA, with post-hoc multiple comparison (Turkey's test). The significant levels were set at $p < 0.05$ and $p < 0.01$.

Results

In this study, a total of 23 primary tooth pulps were analyzed including distribution of the 3 caries subgroups: 1) no caries or caries within enamel (i.e. intact dentin, *n* = 6), 2) caries progress within dentin (dentin exposed, *n* = 9) and 3) caries exposed pulp (pulpal exposed, *n* = 8) and 3 different areas: 1) the pulp horn, 2) the occlusal subodontoblastic region and 3) the midcoronal pulp region (Figure 1) were quantitatively analyzed:

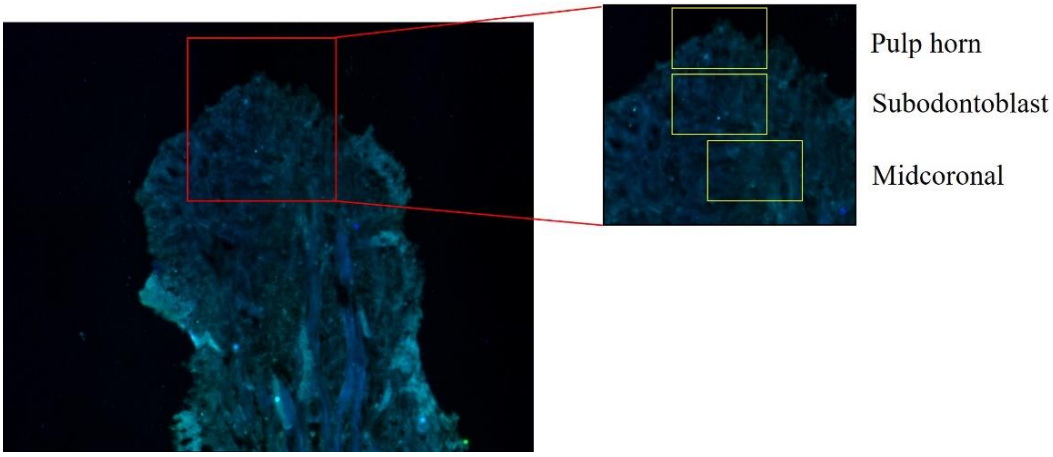

Figure 1. Immunofluorescent image showing areas subject to quantitative analysis within the coronal pulp.

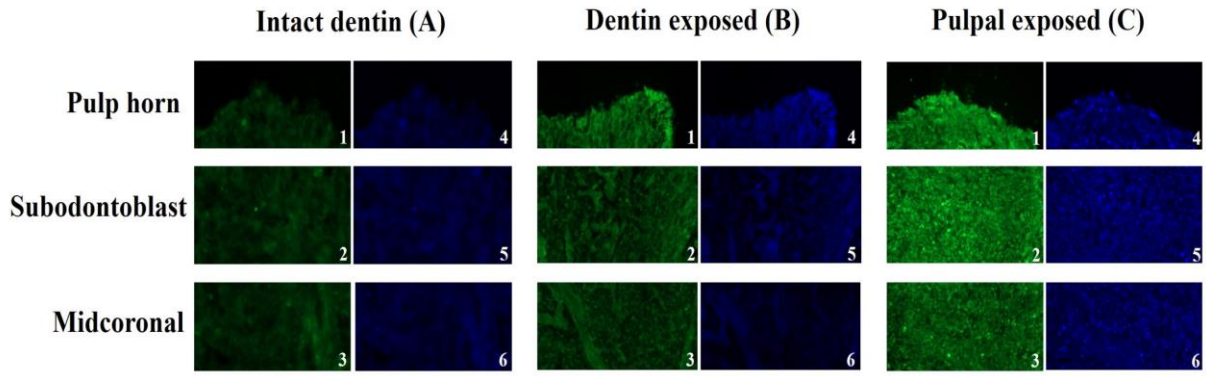

Figure 2. Immunofluorescent image showing TRPA1 (green staining) and TRPV1 (blue staining) in dental pulp within degree of caries progression at 3 area of interest; Representative images of TRPA1 (**A1-3**) and TRPV1 (**A4-6**) of intact dentin group at pulp horn, subodontoblast and midcoronal. Representative images of TRPA1 (**B1-3**) and TRPV1 (**B4-6**) of dentin exposed group at pulp horn, subodontoblast and midcoronal. Representative images of TRPA1 (**C1-3**) and TRPV1 (**C4-6**) of pulpal exposed group at pulp horn, subodontoblast and midcoronal.

Table 1. Mean ± SD intensity of staining for TRPA1 and TRPV1 according to carious progression.

Area of Interest	Degree of caries progression			*p* value
	Intact dentin	Dentin exposed	Pulpal exposed	
TRPA 1				
Pulp horn	1009.950 ± 145.208	1237.934 ± 248.526	1918.313 ± 449.324	**< 0.001 ****
Subodontoblast	916.381 ± 104.36	1076.370 ± 164.184	1755.311 ± 393.507	**< 0.001 ****
Midcoronal	923.559 ± 97.047	999.947 ± 118.886	1826.122 ± 365.233	**< 0.001 ****
***p* value**	**0.3345**	**0.0348 ***	**0.7246**	
TRPV1				
Pulp horn	808.291 ± 221.046	725.674 ± 207.190	895.406 ± 184.157	**0.2517**
Subodontoblast	793.703 ± 258.300	656.844 ± 177.412	828.143 ± 180.604	**0.2081**
Midcoronal	796.623 ± 263.60	703.352 ± 169.998	939.449 ± 88.417	**0.0414 ***
***p* value**	**0.9942**	**0.7276**	**0.3797**	

* Significant difference at P < 0.05
** Significant difference at P < 0.01

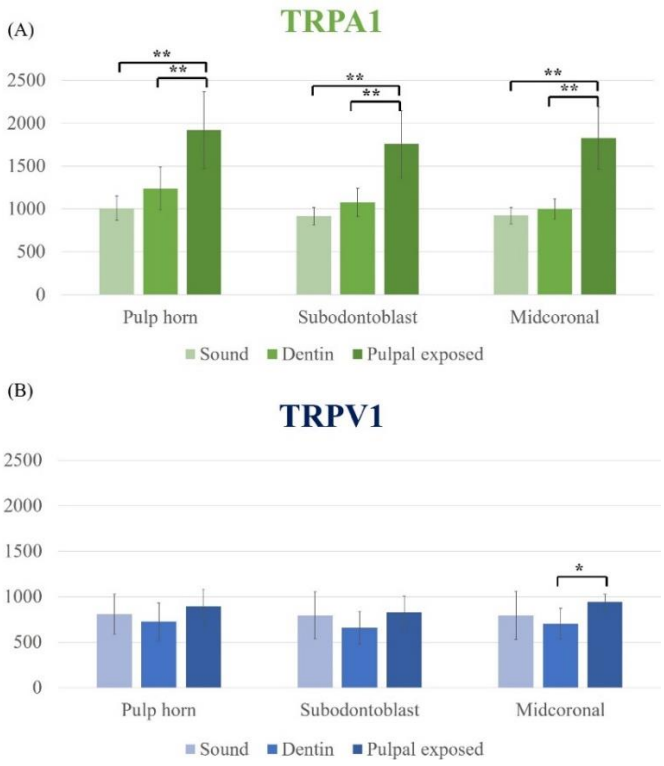

Figure 3. Bar charts showing mean ± SD intensity of staining for TRPA1 and TRPV1. *(A)* represents TRPA1 in pulp horn, subodontoblast and midcoronal of each degree of caries. *(B)* represents TRPV1 in pulp horn, subodontoblast and midcoronal of each degree of caries. * $p < 0.05$. ** $p < 0.01$.

1. TRPA1 and TRPV1 densely expression in the pulp horn more than subodontoblast and midcoronal region

Our study demonstrated the areas of TRPA1 (green) and TRPV1 (blue) staining in three interesting regions and the degree of caries progression. In each degree of caries progression, both TRPA1 and TRPV1 had the most intense staining at pulp horn, subodontoblast and midcoronal area respectively. But significant difference was found only in TRPA1's mean intensity of dentin exposed group between pulp horn and midcoronal area ($p < 0.05$) as show in Table 1.

2. TRPA1 expression increased following caries progression

The intensity of TRPA1 in the same area of interest increased when caries involved dentin and pulpal exposed. There were significant differences between pulpal exposed group with intact dentin and dentin exposed group in all areas ($p < 0.01$), but no significant difference between intact dentin and dentin exposed group.

3. TRPV1 expression decrease when caries progress into dentin then increases when caries involved pulp

The intensity of TRPV1 in the same area of interest decrease when caries progress into dentin, in contrast increase once caries exposed pulp. But only intensity of dentin exposed and pulpal exposed group at midcoronal are significant difference ($p < 0.05$).

Discussion

This study sought to identify the expression of TRPV1 and TRPA1 within intact and carious tooth pulps by employing immunofluorescent staining. Immunolabelling for these 2 antigens permitted good visualization of pulpal neuronal responses and revealed the presence of two different subpopulations.

In primary tooth pulp, this study has shown that TRPA1 and TRPV1 found dominantly in the pulp horn region. Although, there was the evidence of TRPA1 upregulated following the progression of the carious lesion, it is possible that TRPV1 changes were differed. Since we found the down-regulation of TRPV1 when carious progression limited in dentine, before up-regulated again once the pulp was exposed.

It is possible, by virtue of the greater number of resident leucocytes present in primary tooth pulp, that inflammatory cell responses may be more rapidly mounted in the primary dentition [15]. TRPA1 showed an overall significant increase in pulpal exposed groups. These may be influenced from the effects of immune cell proliferation and migration that are

fundamental to the body's defence mechanisms and have been well described in the tooth pulp following caries [2]. However, there was no significant difference in TRPV1 between each degree of caries progression. From the study of Rodd demonstrated the increase of TRPV1 following the progression of carious lesion in permanent dentition [16], but we depict this phenomenon in the primary dentition.

Over the past decade, there has been increasing interest in immune cell and neural interactions, particularly in relation to neurogenic inflammation. The observations made in this study appear to support the complexity of neural and immunal relationships within the dental pulp.

Conclusion

The expression of TRPA1 up-regulated following caries progression but TRPV1 express down-regulated when caries progress within dentin and up-regulated once caries involved pulp to defense against carious progression.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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P006

Effect of *Moringa oleifera* on Nitric Oxide Production in Endothelial Cells

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ABSTRACT

Introduction: *Moringa oleifera* is a plant that has been commonly used for food and traditional medicine. Recently, the effects of this plant related to the cardiovascular system have been studied. This plant can reduce high blood pressure in L-NAME-induced hypertensive rats. However, the effect of *Moringa oleifera* leaf extract (MOE) on endothelial nitric oxide synthase (eNOS) has not yet been reported.

Objectives: We aimed to examine the effect of MOE on nitric oxide (NO) production and eNOS phosphorylation, a marker of eNOS activation, in endothelial cells.

Methods: MOE at 5 and 50 µg/mL were added to the primary human pulmonary artery endothelial cells (HPAECs). After incubation for 5, 10, and 30 min, cell lysates were collected. The cellular NO was measured by chemiluminescence. In the separated set of experiments, HPAECs were treated with 50 µg/mL MOE for 1, 3, 5, 10, and 30 min to investigate the MOE effect on eNOS phosphorylation. The cell lysate was collected, and eNOS phosphorylation was measured by western blot.

Results: After incubations for 5, 10, and 30 min, MOE at 50 µg/mL increased the cellular NO in HPAECs compared with control untreated cells. MOE at 5 µg/mL could increase NO production only after incubation for 30 min. Quercetin (50 µM) and ACh (100 µM) increased the cellular NO in HPAECs. MOE at 50 µg/mL increased eNOS phosphorylation at Ser^{1177}.

Conclusion: MOE can increase NO production by HPAECs through eNOS phosphorylation.

Keywords: *Moringa oleifera*, nitric oxide, eNOS, endothelial nitric oxide synthase, endothelial cell

Introduction

Nitric oxide (NO) is a molecule with many physiological and pathological activities, including vasodilation, angiogenesis, and platelet inhibition [1]. Impaired NO-mediated vasodilation is associated with the pathogenesis of vascular diseases, such as hypertension [2]. NO induces vasodilation by activating soluble guanylyl cyclase to convert guanosine triphosphate to cyclic guanosine monophosphate (cGMP). The cGMP leads to the activation of protein kinase G, which stimulates myosin phosphatase resulting in smooth muscle relaxation [3].

Moringa oleifera is a plant that is native to Asia and widely found in Thailand. The leaves of *Moringa oleifera* are used as food supplements and medicine. MOE from leaves can reduce blood pressure in hypertensive rats induced by L-NAME (an NO synthase inhibitor) [4]. The mechanism of MOE on blood pressure reduction in rats was endothelial-dependent. However, the effect of MOE on eNOS activity has never been reported.

eNOS contributes to the majority of circulating NO. This enzyme synthesizes NO in response to several stimuli, such as acetylcholine, bradykinin, and shear stress (5). In this study, we hypothesized that MOE could increase NO production via eNOS phosphorylation. We used an *in vitro* culture model with HPAECs and measured the cellular NO by chemiluminescence and eNOS phosphorylation by western blot. Also, we used quercetin as a possible active compound in MOE because of its ability to activate eNOS [5, 6].

Methods

1. Extract preparation

Moringa oleifera leaves were collected from Kon Kaen Province, Thailand, and sun-dried for one day. The leaves were chopped and boiled in water (100 g of leaves per 1 L of water) for 40 min. The solution was filtered through cotton and gauze. The aqueous suspension went through a rotary evaporator and lyophilized, resulting in MOE powder. The yield of MOE was 4.975% (w/w). The powder was stored at 4 ^{o}C.

2. Cell culture

HPAECs were purchased from the American Type Culture Collection (ATCC, PCS-100-022; Manassas, VA, USA). HPAECs were cultured in basal vascular medium (ATCC, PCS-100-030) supplemented with endothelial cell growth kit-BBE (ATCC, PCS-100-040), 100 UI/mL penicillin, and 100 µg/mL streptomycin (Capricorn Scientific, Ebsdorfergrund, Germany). Cells were kept at 37 ^{o}C in an incubator with 5% CO_2 and were used at passages 4-

8. The cells were used in the experiment when they reached approximately 80% confluence. Cells were plated onto 12-well plate, 150,000 cell/well for NO measurement experiment, and 60 mm disc, 300,000 cell/disc for protein measurement experiment.

3. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Cells were seeded into 96-well plates. After cells were attached on the plates, different concentrations of MOE (0.05-2 mg/mL) and quercetin (0-350 µM) dissolved in fresh media and DMSO, respectively, were added to replace the old media in each well. Cells were incubated for 24 hours. MTT was added, and cells were incubated for 3 h. MTT was replaced with DMSO, and the absorbance of the sample was measured at wavelength 562 nm using a microplate reader (Synergy Neo2, Biotek, Vermont, USA).

4. Treatment of HPAECs with MOE

Cells plated on 12-well culture plates were treated with MOE at 5 and 50 µg/mL, or acetylcholine (100 µg/mL), a known NO production stimulator as a positive control, and quercetin (50 µM), one of the components found in MOE. Cells were incubated for 5, 10, 30 and 60 min. Treated HPAECs were washed with nitrite-free cold PBS. Cells were added with lysis buffer and scraped off. Cell lysate was collected and kept at -80 ^{o}C.

5. Measurement of the cellular NO

Cell lysates were thawed on ice and vortexed. Afterward, the cell lysates were injected into the purge vessel containing tri-iodide connected to the chemiluminescence NO analyzer (ANALYZER CLD 88, ECO MEDICS, Duernten, Switzerland) [6]. The area under the curve (AUC) was recorded from the signals.

6. Determination of eNOS phosphorylation in HPAECs

Western blot was used to determine eNOS phosphorylation in HPAECs. After thawing, the cell lysates were measured for protein quantity and loaded into the SDS-PAGE gel. The samples were run and transferred onto the nitrocellulose membrane [7]. Western blot analysis for each specific protein was carried out using the following antibodies: mouse anti-phospho-eNOS (Ser1177), mouse anti-total eNOS, and rabbit anti-actin.

7. Statistical analysis

Data are means and standard deviation (SD). Comparisons were made by Student's t-test or one-way ANOVA followed by Tukey's test. The analysis was done using Graphad Prism software, version 5. *p* value less than 0.05 was assigned statistical significance.

Results

1. Effect of MOE and quercetin on the viability of endothelial cell

We studied the cytotoxicity effect of MOE on endothelial cells by MTT assay. HPAECs were treated with various concentration of MOE (0.05-5 mg/mL) for 24 h. Cell viability was decreased at 3 mg/mL MOE (Figure 1A). In the following experiments, we used MOE at the non-cytotoxic concentrations of 0.005 mg/mL (5 µg/mL) and 0.05 mg/mL (50 µg/mL). HPAECs treated with quercetin up to 350 µM showed no change in cell viability (Figure 1B).

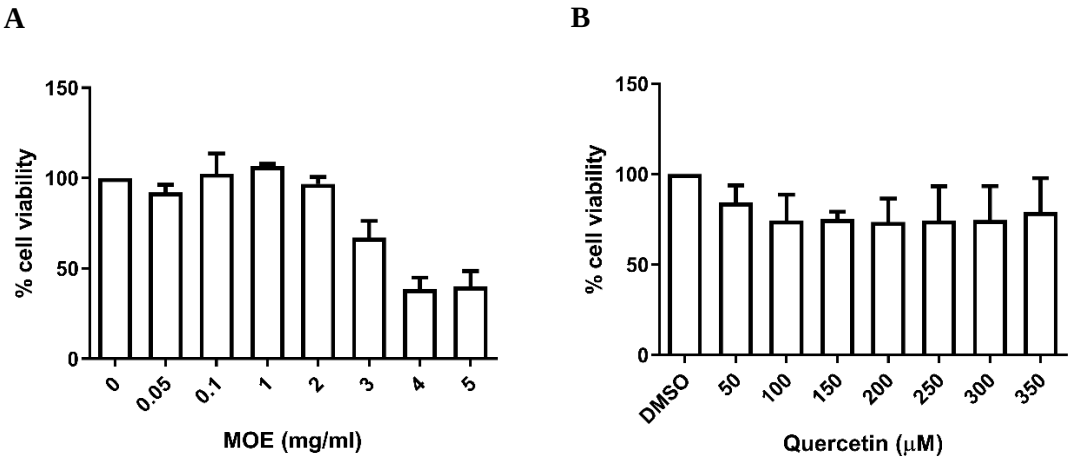

Figure.1 Cell viability of HPAECs after treatment with *Moringa oleifera* extract (MOE) (**A**) or quercetin (**B**). Cells were treated with MOE or quercetin for 24 h before the MTT assay. Each bar represents the mean ± SD from three separate experiments.

Effect of MOE and quercetin on NO production by endothelial cells

We examined the effect of MOE on NO production in HPAECs using acetylcholine as a positive control. Treatment of HPAECs with 100 µM acetylcholine and 50 µg/mL MOE for 5 min increased cellular NO to 0.518 ± 0.245 nmol/mg protein and 0.574 ± 0.081 nmol/mg protein, respectively (Figure 2A). In contrast, the untreated control cells had the cellular NO of 0.235±0.015 nmol/mg protein.

After 10 min incubation, MOE at 50 µg/mL increased the cellular NO in HPAECs to 0.833 ± 0.322 nmol/mg protein (Figure 2B). The untreated control cells had the cellular NO of 0.333 ± 0.205 nmol/mg protein.

After 30 min incubation, MOE at 5 and 50 µg/mL increased the cellular NO in HPAECs to 0.491 ± 0.156 and 0.638 ± 0.184 nmol/mg protein, respectively (Figure 2C). At 30 min, quercetin at 50 µM increased the cellular NO in HPAECs to 0.418 ± 0.083 nmol/mg protein, as compared to the control value of 0.294 ± 0.067 (p = 0.03).

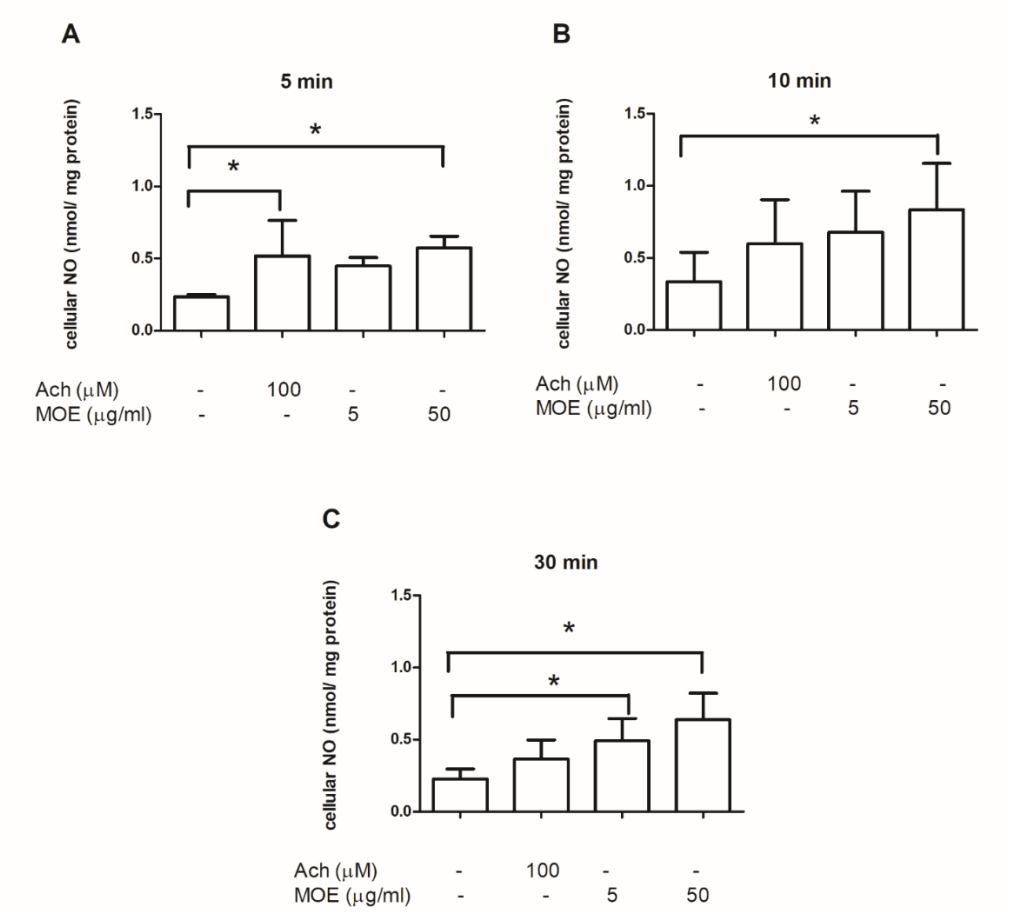

Figure 2. Cellular NO levels in HPAECs treated with *Moringa oleifera extract* (MOE) for 5 (**A**), 10 (**B**), and 30 (**C**) min. Acetylcholine (Ach) at 100 μM was used as a positive control. Each bar represents the mean ± SD from five separate experiments. *$p < 0.05$ by one-way ANOVA followed by Tukey's test.

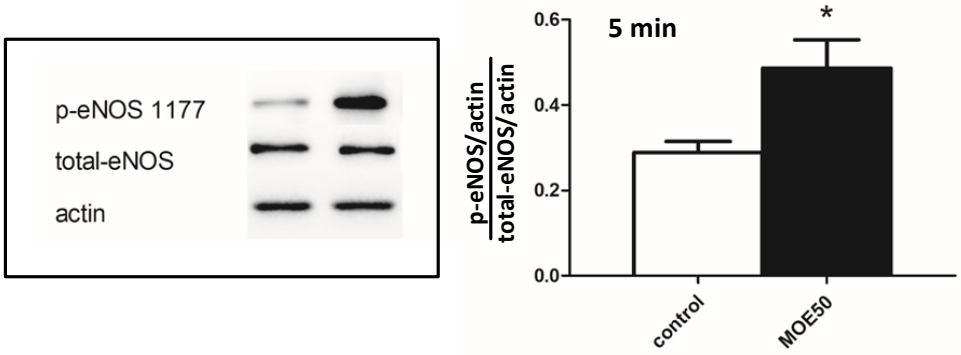

Figure 3. Phosphorylation of eNOS at Ser^{1177} in HPAECs. HPAECs were treated with 50 μg/mL *Moringa oleifera* extract (MOE) for 5 min. The protein lysates were used for western immunoblotting. Each bar represents mean ± SD from three separate experiments. *$p < 0.05$ by Student's t-test.

Effect of MOE on eNOS phosphorylation of HPAEC

Incubation of MOE at 50 µg/mL for 5 min increased eNOS phosphorylation at Ser^{1177} in HPAECs (Figure 3).

Discussion

A previous study on cytotoxicity of MOE on endothelial cells showed that MOE up to 0.3 mg/mL (300 µg/mL) was not toxic to HPAECs [4]. Quercetin at 30 µM did not affect the cell viability of human brain microvascular endothelial cells [8]. The results of previous studies were consistent with our data. In our research, MOE at concentrations up to 2 mg/mL and quercetin at 350 µM were not toxic to HPACEs. Moreover, MOE (10-30 µg/mL) could increase NO production of HPAECs after 30 and 60 min of treatment [4]. Here, we provide further data that 50 µg/mL MOE promotes NO synthesis at 5, 10, and 30 min and eNOS phosphorylation in HPAECs.

We used quercetin as a possible active compound in MOE because quercetin could induce eNOS phosphorylation at Ser^{1179} in bovine aortic endothelial cells (BAECs) [9]. In our study, MOE at 50 µg/mL, which approximately contains 0.03 µM quercetin, could increase NO production. In contrast, pure quercetin at a high concentration (50 µM) increased NO production in HPAECs. Further study is needed to investigate the effect of quercetin at different concentrations on NO production in HPACEs. Other candidate compounds in MOE, such as isoquercetin or catechin [10, 11], should also be tested.

Our findings suggest that the blood pressure-lowering of *Moringa oleifera* is via the increased NO production and eNOS activity in endothelial cells.

Conclusion

MOE can increase NO production in HPAECs via increasing eNOS phosphorylation.

Conflict of interest

The authors declare that they have no conflicts of interest.

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ผู้สนับสนุนงานประชุมวิชาการประจำปี สมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 42

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19 – 21 พฤษภาคม 2564

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8.	รองศาสตราจารย์รัตติมา จีนาพงษา	กรรมการ
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10.	รองศาสตราจารย์ชลภัทร สุขเกษม	กรรมการ
11.	รองศาสตราจารย์ณัฐวุธ สิบหมู่	กรรมการ
12.	รองศาสตราจารย์พรพรรณ วิวิธนาภรณ์	กรรมการ
13.	รองศาสตราจารย์ลัดดาวัลย์ เส็งกันไพร	กรรมการ
14.	รองศาสตราจารย์สุพีชา วิทยเลิศปัญญา	กรรมการ
15.	รองศาสตราจารย์สุวรา วัฒนพิทยกุล	กรรมการ
16.	รองศาสตราจารย์ศุภนิมิต ทีฆชุณหเถียร	กรรมการ
17.	รองศาสตราจารย์ศิวนนท์ จิรวัฒโนทัย	กรรมการ
18.	รองศาสตราจารย์วรรณา ชัยเจริญกุล	กรรมการ
19.	ผู้ช่วยศาสตราจารย์วัชรี ลิมปนสิทธิกุล	กรรมการ
20.	ผู้ช่วยศาสตราจารย์วันดี อุดมอักษร	กรรมการ
21.	ผู้ช่วยศาสตราจารย์สกลวรรณ ประพฤติบัติ	กรรมการ
22.	ผู้ช่วยศาสตราจารย์ศริญญา คงเพชร	กรรมการและเลขานุการ
23.	อาจารย์นนทญา นาคคำ	กรรมการและผู้ช่วยเลขานุการ

7. คณะกรรมการฝ่ายการเงิน และลงทะเบียน

1.	รองศาสตราจารย์เอื้อมเดือน ประวาฬ	ประธานกรรมการ
2.	ผู้ช่วยศาสตราจารย์ศริญญา คงเพชร	กรรมการ
3.	นายธนวัช แก้วคำสอน	กรรมการ

4. อาจารย์คัมภีร์พร บุญหล่อ กรรมการและเลขานุการ
5. นางอุมาพร โพธิ์ชัย กรรมการและผู้ช่วยเลขานุการ
6. นางสาวอารีรัตน์ ดรเสนา กรรมการและผู้ช่วยเลขานุการ

8. คณะกรรมการฝ่ายต้อนรับ พิธีการ สันทนาการ และกิจกรรม

1. ผู้ช่วยศาสตราจารย์ศิริมาศ กาญจนวาศ ประธานกรรมการ
2. ผู้ช่วยศาสตราจารย์ศิริพร เทียมเก่า กรรมการ
3. ผู้ช่วยศาสตราจารย์ปณต ตั้งสุจริต กรรมการ
4. อาจารย์นนทญา นาคคำ กรรมการ
5. อาจารย์คัมภีร์พร บุญหล่อ กรรมการ
6. อาจารย์พัทธมน ลพานุวรรตน์ กรรมการ
7. อาจารย์สรายุทธ หลานวงศ์ กรรมการและเลขานุการ

9. คณะกรรมการฝ่ายประชาสัมพันธ์และไอที

1. ผู้ช่วยศาสตราจารย์ปณต ตั้งสุจริต ประธานกรรมการ
2. อาจารย์สรายุทธ หลานวงศ์ กรรมการและเลขานุการ
3. นายธนวัช แก้วคำสอน กรรมการและผู้ช่วยเลขานุการ
4. นายวิโรจน์ รอดสอน กรรมการและผู้ช่วยเลขานุการ

10. คณะกรรมการฝ่ายหารายได้

1. ผู้ช่วยศาสตราจารย์ศิริมาศ กาญจนวาศ ประธานกรรมการ
2. รองศาสตราจารย์ลัดดาวัลย์ เส็งกันไพร กรรมการ
3. ผู้ช่วยศาสตราจารย์คัชรินทร์ ภูนิคม กรรมการ
4. ผู้ช่วยศาสตราจารย์ศิริพร เทียมเก่า กรรมการ
5. ผู้ช่วยศาสตราจารย์ดนุ เกษรศิริ กรรมการและเลขานุการ
6. นางอุมาพร โพธิ์ชัย กรรมการและผู้ช่วยเลขานุการ

11. คณะกรรมการฝ่ายสถานที่ อาหาร ยานพาหนะ และ โสตทัศนูปกรณ์

1. ผู้ช่วยศาสตราจารย์ปณต ตั้งสุจริต ประธานกรรมการ
2. อาจารย์สรายุทธ หลานวงศ์ กรรมการ
3. นายธนวัช แก้วคำสอน กรรมการ
4. นางสาวอารีรัตน์ ดรเสนา กรรมการ
5. นายวิโรจน์ รอดสอน กรรมการ
6. นางสุลิพร ปานพรม กรรมการและเลขานุการ
7. นางเอื้อสุข พรมลือชัย กรรมการและผู้ช่วยเลขานุการ

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