PROCEEDINGS

The 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand

Frontiers in Precision Medicine and Targeted Therapy

24-26 May 2023

The Rembrandt Hotel Bangkok

THE PHARMACOLOGICAL AND THERAPEUTIC SOCIETY OF THAILAND

Interdisciplinary Pharmacology

This publication includes abstracts of the invited speakers and poster abstracts and papers presented at the 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand under the theme "Frontiers in Precision Medicine and Targeted Therapy". The conference was held at the Rembrandt Hotel, Bangkok from May 24-26, 2023, organized by Interdisciplinary Program in Pharmacology, Graduate School, Chulalongkorn University, Bangkok, Thailand.

Disclaimer
Matters of copyright of all images and text associated with the abstracts and papers within the Proceedings of the 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand (PTST) are the responsibility of the authors. The PTST does not accept responsibility for any liabilities arising from the publication of any of the submissions.

Copyright
Reproduction of this document or parts thereof by any means what soever is prohibited without the written permission of the Pharmacological and Therapeutic Society of Thailand (PTST).

National Library of Thailand Cataloging in Publication Data

Proceedings of the 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand. -- Bangkok : The Pharmacological and Therapeutic Society of Thailand, 2023.
66 p.

1. Pharmacology. 2. Therapeutics. I. Title.

615.1
ISBN 978-616-94260-0-4

Table of Contents

Symposium III

Symposium IV

Poster Abstracts

Poster Papers

Welcome Message from the Organizing Committee Chair

Dear Colleagues

It is our great pleasure to welcome you to the 44th Pharmacological and Therapeutic Society of Thailand Meeting: **"Frontier in Precision Medicine and Targeted Therapy"** to be held in Bangkok during May 24-26, 2023. This meeting has been organized on-site for the first time in 2 years after the COVID-19 pandemic shut-down and is an opportunity for all participants to attend the meeting in person. In order to expand our roster of outstanding speakers, presentations in this meeting will be hybrid of teleconference and on-site ones. We ask for your patience should we have any technical issues from this arrangement.

This 44th annual meeting upholds a long legacy and reputation of the Pharmacological and Therapeutic Society of Thailand in strengthening the up-to-date knowledge and future challenges in pharmacological sciences. This meeting features a scientific program in precision medicine and targeted therapy, particularly in pharmaco-genomics, cardiovascular diseases, cancer, senescence and immunotherapy, clinical pharmacokinetics, and gene and cell therapy. Equally important, the meeting is an excellent platform for networking and collaboration in the pharmacologists' community. Hopefully, all attendees can benefit from this meeting.

Finally, we would like to express our heartfelt gratitude to all the assistants and staff who have been working diligently to make this meeting possible. Our sincere appreciation also goes to the committee of the Pharmacological and Therapeutic Society of Thailand for granting and supporting this meeting.

Associate Professor Suree Jianmongkol, PhD
Chair of the Organizing Committee

Welcome Message from the President of PTST

Dear PTST members and all participants,

The 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand is organized by Chulalongkorn University (Multidisciplinary Pharmacology Program) during 24-26 May 2023 under the theme of **"Frontiers in Precision Medicine and Targeted Therapy"**. The meeting venue is the Rembrandt Hotel Bangkok. I am pleased to welcome all PTST members and participants to this first on-site meeting since the COVID-19 pandemic in 2019. The conference focuses on advanced knowledge and technologies in precision medicine, which covers pharmaco-genomics and pharmacometrics, particularly in important diseases such as cardiovascular diseases, cancer, and infectious diseases. This 3-day conference consists of plenary lectures, symposiums, poster presentations and laboratory visits.

It is a great opportunity and exciting for the researchers in pharmacology and related fields to meet on-site again after three years. I am pleased that the Journal of Basic and Applied Pharmacology (JBAP) can be a platform for researchers and students to publish their work.

Kesara Na-Bangchang
Professor in Pharmacology
President of of the Pharmacological and Therapeutic Society of Thailand (PTST)

Wednesday 24th May 2023	
8.30-9.00	Registration
9.00-9.30	Welcome ceremony
9.30-10.30	**Chiravat Sadavongvivad's memorial lecture** **The power of pharmacogenomics in precision medicine** Professor Wichittra Tassaneeyakul, Faculty of Medicine, Khon Kaen University
10.30-10.45	Coffee break
10.45-11.45	**Plenary lecture 1** **Decoding the human blood-brain barrier logistics and its relevance to drug delivery to the brain and targeted therapy of brain disease** Professor Masanori Tachikawa Graduate School of Biomedical Sciences, Tokushima University, Japan (Under collaboration between The Pharmacological and Therapeutic Society of Thailand and The Japanese Society for the study of Xenobiotics (JSSX)) (tele-conference)
11.45-12.15	Pharmacological and therapeutic society of Thailand's award ceremony
12.15-13.00	Lunch Symposium 1: Biodesign Co., Ltd.
Symposium 1: Pharmacogenomics	
13.00-14.00	**Patient-friendly stratified drug therapy based on pharmacogenomic testing** Professor Taisei Mushiroda Head of Laboratory for Pharmacogenomics, Center for Integrative Medical Sciences, The Institute of Physical and Chemical Research (RIKEN), Japan (tele-conference)
14.00-14.45	**HLA genotypes with highly accurate long-read SMRT sequencing and its association to anti-rHu-EPO-associated pure red cell aplasia** Assistant Professor Pajaree Chariyavilaskul Faculty of Medicine, Chulalongkorn University
14.45-15.30	Coffee break and Poster presentation
Symposium 2: Cardiovascular disease	
15.30-16.30	**New mechanisms of drug used to treat patients with cardio-metabolic disease** Professor Yu Huang City University of Hong Kong, Hong Kong (tele-conference)
16.30-17.15	**Biomarkers and targeted therapy for cardiac fibrosis: Pathogenesis, diagnosis and therapeutic approaches** Professor Supachoke Mangmool Faculty of Science, Mahidol University
17.15-18.00	Annual Member Meeting of the Pharmacological and Therapeutic Society of Thailand

Thursday 25th May 2023	
8.00-8.30	Registration
8.30-9.30	Plenary lecture 2 **Crafting Originals via TechBio Platforms** Dr. Trairak Pisitkun Faculty of Medicine, Chulalongkorn University
9.30-9.45	Coffee break
Symposium 3: Cancer, Senescence and immunotherapy	
9.45-10.45	**Understanding of the host adaptive mechanism of cancer cells through immune-editing process and its application for cancer immunotherapy** Professor Yoshihiro Hayakawa Toyama University, Japan (onsite)
10.45-11.30	**The role of DNA protection of HMGB1-produced DNA gap (REDGEM) in the reversal of irreversible senescence-associated diseases and conditions** Professor Apiwat Mutirangura Faculty of Medicine, Chulalongkorn University
11.30-12.15	**Molecular pharmacology and drug targets for anti-cancer drug discovery** Professor Pithi Chanvorachote Faculty of Pharmaceutical Sciences, Chulalongkorn University
12.15-13.00	Lunch Symposium 2: Bio-Active Co., Ltd.
Symposium 4: Clinical pharmacokinetic	
13.00-14.00	**Model Informed Precision Dosing for the Personalized Pharmacotherapy** Professor Jae Gook Shin Inje University College of Medicine, Busan, South Korea (tele-conference)
14.00-14.45	**Clinical applications of PK/PD in pediatric antimicrobial drug dosing** Assistant Professor Noppadol Wacharachaisurapol Department of Pharmacology, Faculty of Medicine, Chulalongkorn University
Symposium 5: Gene and cell therapy	
14.45-15.45	**Current use and promising future of gene therapy in Thailand** Associate Professor Oranee Sanmaneechai Faculty of Medicine Siriraj Hospital, Mahidol University
15.45-16.30	**Development of sustainable cellular therapy for blood cancer in Thailand** Dr. Koramit Suppipat Faculty of Medicine, Chulalongkorn University
16.30-17.30	Best poster awards and closing ceremony

Friday 26th May 2023	
8.30-9.00	Registration
9.00-12.00	**Plant-based production of biopharmaceuticals** Associate Professor Suthira Taychakhoonavudh Chief Executive Officer & Co-Founder and Kitti Jirananon, Pharmacist (Plant director) Baiya Phytopharm Co., Ltd. or **Non-clinical efficacy and safety studies for pharmaceuticals** Associate Professor Anusak Kijtawornrat Director of Chulalongkorn University Laboratory Animal Center (CULAC)

Chiravat Sadavongvivad's Memorial Lecture

The Power of Pharmacogenomics in Precision Medicine

Wichittra Tassaneeyakul

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

E-mail: wichittra.tassaneeyakul@gmail.com

Abstract

Inter-individual differences in response to drugs, both in terms of efficacy and safety, are common and may become a growing issue globally due to the increasing aging population requiring drug therapy. Genetic factors are a significant contributor to such variability. With the increasing availability and decreasing cost of genetic technologies, implementing pharmacogenomics in clinical practice is widely recognized as an essential step in precision medicine. By conducting pharmacogenetic testing, healthcare professionals can identify patients who are more likely to respond to specific drugs, enabling them to prescribe the most effective treatment from the beginning. Additionally, pharmacogenetic testing can help identify patients at higher risk of experiencing adverse drug reactions, allowing physicians to adjust dosages or prescribe alternative drugs as necessary. Furthermore, pharmacogenomics can assist pharmaceutical companies in identifying which patient subgroups are most likely to respond to a particular drug, facilitating the development of more efficient and cost-effective clinical trials. Lastly, it can also be utilized for drug discovery and development, assisting in the identification of new drug targets. This lecture will provide examples of the use of pharmacogenomics in clinical practice as well as drug development.

Keywords: Pharmacogenomics, precision medicine, drug development

Plenary Lecture I

Decoding, Building, and Manipulating of the Human Blood-Brain Barrier: Perspectives on Brain-targeting Drug Delivery Systems

Masanori Tachikawa

Graduate School of Biomedical Sciences, Tokushima University, Japan

E-mail: tachik-dds@umin.ac.jp

Abstract

The number of patients with neurological disorders and neurodevelopmental disorders has been increasing worldwide. These central nervus system (CNS) diseases significantly reduce the patients' quality of life and so novel CNS-acting drugs are much anticipated. Attracting attentions are now being paid to development of macromolecular drugs such as enzymes, antibodies, and nucleic acids for the CNS therapeutics. However, the blood-brain barrier (BBB), which is formed by brain microvasculature network, is an impregnable obstacle for brain-targeting drug delivery. This would be a reason for the low success rate of human clinical trials for CNS therapeutics. It is thus important to establish the novel brain-targeting delivery systems of the macromolecular drugs at the BBB. To achieve the goal, we are devising three research streams of decoding, building, and manipulating of the human BBB.

Emerging evidence has shown that the BBB possesses a variety of transport systems such as transporters, channels, and receptors, so called "the BBB logistics", to transfer nutrients, essential proteins, nucleic acids-containing extracellular vesicles, and cells from the circulating blood to the brain. From this viewpoint, decoding the human BBB logistics at the molecular levels would lead to discovery of the new drug delivery route at the BBB. We have created a quantitative protein atlas of the human BBB transport systems using quantitative targeted absolute proteomics (*Cells* 11:3963, 2022). We have also succeeded in reconstructing a three-dimensional human brain microvasculature model on a microfluidic device for predicting the human BBB permeability. This is being conducted as a project of "building the human BBB on-a-chip" which can mimic the BBB logistics. We are now trying to manipulate the BBB transport systems using the BBB-penetrating extracellular vesicles. In this symposium, I would like to discuss how our knowledge can be applied to the development of the brain-targeting drug delivery systems.

Keywords: Blood-brain barrier, logistics, quantitative proteomics, microfluidics, extracellular vesicles

Invited Speaker (Symposium I)

Patient-Friendly Stratified Drug Therapy Based on Pharmacogenomic Testing

Taisei Mushiroda

Laboratory for Pharmacogenomics,
RIKEN Center for Integrative Medical Sciences, Japan

E-mail: mushiroda@riken.jp

Abstract

Adverse drug reactions (ADRs) such as skin rash, drug-induced liver injury, and agranulocytosis have long been analyzed in relation to human leukocyte antigen (HLA) that is an important molecule involved in human immunity. HLA is composed of many genes, each of which has dozens of different types (alleles), and many HLA alleles associated with ADRs have been reported. The odds ratios in the association with HLA alleles range from approximately 5 to several thousand, indicating a very large impact on the risk of ADRs. Since it is necessary to prospectively show the effects of medical interventions based on the genectic test results, we conducted GENCAT study, a prospective, multicenter, single-arm clinical trial to investigate the impact of a therapeutic intervention based on the HLA-A*31:01 test on the incidence of carbamazepine-induced skin rash. HLA-A*31:01-positive patients were treated with an alternative drug such as valproic acid, and the study showed an approximately 60% reduction in the incidence of carbamazepine-induced skin rash.

As indicated by the fact that drug-metabolizing enzymes and transporters account for approximately 50% of the 310 germline PGx biomarkers described in the U.S. drug inserts, it is important to evaluate pharmacokinetics (PK)-related genetic variants in the realization of stratified medicine based on pharmacogenomics. We have developed a comprehensive targeted next-generation sequencing (NGS) analysis panel, PKseq, for 100 PK-related genes, including 62 drug-metabolizing enzymes and 37 drug transporters. Sequencing of the 100 genes using PKseq resulted in improved accuracy compared to whole genome sequencing (WGS) and whole exome sequencing (WES), as well as reduced cost, time, and analysis burden. PKseq will become a powerful tool for discovery research on common and rare variants that cause inter-individual differences in drug efficacy and ADRs, and clinical implementation of pharmacogenomics testing.

Keywords: HLA, carbamazepine, clinical utility, CYP2D6, NGS

Invited Speaker (Symposium I)

HLA Genotypes with Highly Accurate Long-Read SMRT Sequencing and Its Association to Anti-rHu-EPO-Associated Pure Red Cell Aplasia

Pajaree Chariyavilaskul

Center of Excellence in Clinical Pharmacokinetics and Pharmacogenomics,
Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

E-mail: pajaree.l@chula.ac.th

Abstract

Pure red cell aplasia (PRCA) is a rare hematological disorder characterized by the absence of erythropoiesis, the process by which red blood cells are produced. The most common cause of PRCA is the development of neutralizing antibodies against erythropoietin (EPO), a hormone that regulates erythropoiesis. These antibodies interfere with the action of EPO, leading to a decrease in red blood cell production and resulting in anemia. PRCA can also occur as a side effect of EPO therapy in patients with chronic kidney disease, as well as in patients with certain types of cancer. Treatment options for PRCA include discontinuation of EPO therapy, immunosuppressive therapy, and blood transfusions. Human leukocyte antigen (HLA) has been found to play a role in the development of PRCA in patients receiving EPO therapy. Studies have shown that certain HLA alleles, such as HLA-A02 and HLA-B44, are associated with an increased risk of developing PRCA. Additionally, it has been observed that patients who develop PRCA while receiving EPO therapy are more likely to have HLA haplotypes that are different from those of the general population. This suggests that HLA may play a role in the immune response to EPO, leading to the development of neutralizing antibodies and resulting in PRCA. However, it is important to note that the exact mechanism by which HLA alleles influence the development of PRCA is not fully understood.

Long read Single Molecule, Real-Time (SMRT) sequencing is a powerful tool that can be used to study the HLA in relation to PRCA caused by EPO therapy. This technique allows for the accurate and comprehensive characterization of the HLA region, which is important as HLA alleles have been found to be associated with an increased risk of developing PRCA. Long read SMRT sequencing can provide ultra-long reads of the HLA region, which allows for the detection of structural variations and copy number variations that may not be captured by other sequencing methods. This can provide a more detailed and accurate picture of the HLA region in patients who develop PRCA while receiving EPO therapy. Additionally, long read SMRT sequencing can also enable the identification of rare HLA alleles, which may be important in understanding the genetic susceptibility to PRCA. This can help to identify potential biomarkers for predicting the risk of PRCA in patients receiving EPO therapy. It's worth noting that, this technology is relatively new and more studies are needed to confirm its clinical utility in PRCA. It's also important to note that the cost of this technology might be high and it is not yet widely adopted in clinical settings.

Keywords: Pure red cell aplasia, erythropoietin, chronic kidney disease, HLA, long-read sequencing

Invited Speaker (Symposium II)

New Mechanisms of Anti-cardio-metabolic Disease Drugs

Yu Huang

Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China

E-mail: yu-huang@cityu.edu.hk

Abstract

Healthy vascular endothelium is the critical player in maintaining vascular homeostasis through releasing several vaso-protective substances called endothelium-derived relaxing factors (EDRFs) such as nitric oxide. By contrast, loss of EDRFs in diseased endothelial cells unmasks the vaso-harmful impact of endothelium-derived contracting factors (EDCFs) such as vasoconstrictive prostanopids. Such disrupted balance between EDRFs and EDCFs in endothelium is referred to endothelial dysfunction, an important initial pathological event that triggers pathogenesis of vascular diseases in hypertension and diabetes. Increased production of reactive oxygen species (ROS) or raised oxidative stress in the vascular wall is probably the key factor to inactivate nitric oxide within endothelial cells. Understanding and targeting the sources of ROS is effective to increase the bioavailability of endothelium-derived nitric oxide, thus improving endothelial function in cardio-metabolic diseases. For example, a number of drugs clinically used to treat cardiovascular and metabolic diseases are able to reduce vascular oxidative stress so as to augment endothelial function in arteries from animals of diseases and from patients. In addition, targeting endothelium is also useful to inhibit vascular inflammation and disturbed blood flow-associated development of atherosclerosis.

Keywords: Endothelial dysfunction, diabetes, hypertension, atherosclerosis

Invited Speaker (Symposium II)

Biomarkers and Targeted Therapy for Cardiac Fibrosis: Pathogenesis, Diagnosis, and Therapeutic Approaches

Supachoke Mangmool

Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand

E-mail: supachoke.man@mahidol.ac.th

Abstract

Cardiac fibrosis contributes to the abnormality of cardiac functions, leading to the development of cardiac remodeling and progression of heart failure (HF). After cardiac injury, cardiac fibroblasts can be induced by many profibrotic mediators (e.g., angiotensin II, endothelin-1, and transforming growth factor-β1) into the activated form, resulting in increased fibroblast proliferation, deposition of extracellular matrix proteins, secretion of cytokines and growth factors. Inhibition of myofibroblast differentiation may serve as an effective means to prevent the progression of fibrosis. However, the molecular mechanisms of these profibrotic mediators on the myofibroblast activation and differentiation is not fully understood.

Cardiac fibroblasts possess the potential to differentiate into myofibroblasts, which are distinguished from fibroblasts by their expressions of many pathological markers, including α-smooth muscle actin and stress fiber formation. Thus, identification of these pathologic markers for myofibroblast activation and differentiation are seemed to be the potential tools for early diagnosis of cardiac fibrosis.

Unfortunately, patients with fibrotic disorders expressed signs and symptoms and reached clinical care after significant fibrosis has already advanced. Interestingly, many studies have reported that myofibroblast differentiation process can be reversed. For these patients, reversal of myofibroblast differentiation (also called dedifferentiation) represents attractive targeted therapeutic strategies to already established myocardial fibrosis. However, factors affecting on the reversibility of myofibroblast are unclear.

Keywords: Cardiac fibroblast, cardiac fibrosis, biomarker of fibrosis, myofibroblast differentiation, reversibility of myofibroblast

Plenary Lecture II

Crafting Originals via TechBio Platforms

Trairak Pisitkun

Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

E-mail: trairak@gmail.com

Abstract

In this talk, we will focus on the power of TechBio platforms in developing unique, original products that can create competitive advantages and deliver high-impact values in the fight against infectious diseases such as COVID-19, influenza, and RSV. By utilizing these platforms to identify and isolate antibodies from elite responders who have recovered from these diseases, we can develop new treatments and therapies that can make a significant difference in the healthcare industry. We will discuss how these original products can lead to more effective and efficient treatments and how they can provide an edge in the competitive healthcare market. The talk will provide an in-depth look into the potential of TechBio platforms in creating innovative medical products that can have a real impact on patient outcomes and the future of the healthcare industry.

Keywords: Human monoclonal antibodies, COVID-19, TechBio

Invited Speaker (Symposium III)

Acquired Resistance Mechanism of Cancer Cells to Anti-tumor Immunity

Yoshihiro Hayakawa

Section of Host Defences, Institute of Natural Medicine, University of Toyama, Toyama, Japan

E-mail: haya@inm.u-toyama.ac.jp

Abstract

While a primary immune resistance of cancer cells might be caused by their low immunogenicity and/or expression of immune-suppressive phenotypes, acquired resistance mechanisms of cancer cells to anti-tumor immunity is unclear. To establish effective cancer immunotherapy, it is important to overcome such cancer cell resistance against anti-tumor immunity. Therefore, in this study, we aim to understand an acquired resistance mechanism of cancer cells to antigen-specific T cells. By using B16 melanoma cell line expressing ovalbumin (B16-OVA) as a model tumor antigen, we established variant cell lines after exposing to the different immunological conditions in vivo. The immune resistant (IMM) cell lines, but not parental B16-OVA or other control cell lines, showed different in vivo behaviour along with losing the expression of model tumor antigen. Moreover, we further found the expression of particular genes were significantly altered in the immune resistant (IMM) cells compared to the parental B16-OVA or other control cell lines. Of particular interest, there is a significant change in the gene expression, which is known to involve in cellular redox responses, and importantly IMM cell lines tend to be more tolerant to oxidative stress responses induced by several different condition including IFN-γ treatment. To further determine a functional role of the redox-related gene (RRG) in protecting the immune-resistant cancer cells from the oxidative stress, we established the B16-OVA cell line overexpressing RRG or IMM cell line knocking down RRG. While the over-expression of RRG in B16-OVA cell line was not solely responsible to inhibit oxidative stress induced intracellular ROS level, RRG over-expression protected from cellular damage induced by oxidative stress. Contrary, the knockdown of RRG in IMM cell line by using shRNA sensitized to oxidative stress responses. Collectively, these results indicate the importance of cellular redox system in cancer cells for acquiring resistance to immunity.

Keywords: Anti-tumor immunity, IFN-γ, oxidative stress

Invited Speaker (Symposium III)

The Role of DNA Protection of HMGB1-Produced DNA Gap REDGEM) in the Reversal of Irreversible Senescence-Associated Diseases and Conditions

Apiwat Mutirangura

Affiliation Center of Excellence in Molecular Genetics of Cancer and Human Diseases, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

E-mail: mapiwat@chula.ac.th, apiwat.mutirangura@gmail.com

Abstract

HMGB1 release causing deprivation of intranuclear HMGB1 and accumulation of DNA damage in the elderly promotes cellular aging. Cellular aging is the core mechanism of degenerative diseases. Inhibiting the upstream cellular aging process may be an approach to cure degenerative diseases. Our research group reported that cells contain naturally occurring DNA gaps to protect DNA, similar to the gaps left between successive rails on a railway track. The DNA gaps, youth-DNA-gaps, are reduced in the old. Reducing the youth-DNA-gaps causes DNA damage accumulation. We identified a gene called HMGB1 functioning as molecular scissors producing DNA gaps. We used gene therapy technology to add the Box A of HMGB1 (Box A) molecular scissors into old rats' cells. The molecular scissors-produced DNA gaps protected DNA, reduced DNA damage, increased DNA durability, and ultimately rejuvenated old rats. In other words, we introduced Box A to strengthen DNA and restore organ structures and functions, which deteriorated due to old age. We named Box A expression plasmid via its role as REDGEM, which stands for **re**juvenating **D**NA by **ge**nomic stability **m**olecule. We successfully used REDGEM to treat two models, naturally aging and *d*-galactose induced, of senile dementia rats. We also have a promising treatment outcome, including evidence of neurogenesis in Rotenone-induced Parkinson's rats. In addition, we will report the high efficacy of REDGEM in treating diabetes wounds, burn wounds, insulin resistance, liver fibrosis, lung fibrosis, and kidney failure. For safety purposes, we finished the test and analysis of REDGEM in baby porcine and premenopausal cynomolgus macaques. In addition, these large animal tests also provided promising outcomes regarding the future drug for wellness improvement of learning, heart function, and muscle function. In conclusion, REDGEM protects DNA and inhibits the upstream process of degenerative disease pathogenesis.

Keywords: REDGEM, degenerative disease, rejuvenation, DNA damage, youth-DNA-gaps

Invited Speaker (Symposium III)

Molecular Pharmacology of Novel Lead Compounds for Targeted Cancer Therapy

Pithi Chanvorachote

Department of Pharmacology and Physiology,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

E-mail: pithi.c@chula.ac.th

Abstract

Advances in the insights regarding cancer cell biology and cell signaling lead to the discovery of novel drug targets for cancer including cell signals in the regulation of cancer cell growth, metastasis, and cancer stem cells. Several molecular targeted compounds have been revealed to have a potential benefit for cancer management via the activities involving specifically interaction with the molecular targets in the cancer cells regulating growth and metastasis.

As c-Myc is an important transcription factor facilitating cell growth, survival, metastasis, and reprograming that have been found to be dysregulated or aberrantly expressed in 70% of human cancers, making it one of the most important human onco-proteins and a promising molecular target for anti-cancer drugs. We investigate the novel compound EMD (*N*, *N*-bis (5-ethyl-2-hydroxybenzyl) methylamine) that can interact and induce c-Myc degradation via ubiquitin-proteasomal mechanism, and the modifications of molecular moieties of their structure provide the insight of pharmacophore important for the c-Myc targeting action. In addition, the modification of the compound structure with the knowledge of SAR offers its better activity against the cancer stem cells population in lung cancer cells. Furthermore, we investigated the effect of several compounds derived form natural products for their potentials in regulation of other cancer signal directing cancer growth and metastasis. Norcycloartocarpin, ovalitenone, and pongol methyl ether, a plant derived pharmacological active agents were demonstrated to interact with Protein kinase B (Akt) molecule in lung cancer cells and inhibit the protein function. The utilizing of molecular docking offers the information of the affinity and binding energy of the compound and the protein with further information useful for the structure design as a lead compound. As cancer is a disease depending on the status of gene alterations that related to the defected cell signaling, inhibition of cancer aggressive signals that specific to the patient's gene information could be the promising way to personalized treatment designed and benefit the clinical outcome.

Keywords: Drug discovery, c-Myc, Akt, EMD, structure-activity relationship (SAR)

Invited Speaker (Symposium IV)

Model Informed Precision Dosing for Personalized Pharmacotherapy

Jae Gook Shin

Inje University College of Medicine, Busan Paik Hospital, Busan, Korea

E-mail: phshinjg@gmail.com

Abstract

It is well known dogma of pharmacology that therapeutic drug response varies among the individual patients with same therapeutic dose regimen. The drug response variation is caused by many factors of individual subjects such as pharmacogenotype, drug interaction, age, body weight renal/hepatic dysfunction, ethnicity etc. In order to implement the clinical practice of precision medicine, we have to predict the phenotype and clinical decision making of personalized prescription from the genotype of an individual, but it is not an easy process in diverse clinical setting. Although we know the genotype to phenotype relationship of many PK related genes, it is not always easy to validate the clinical relevance of genetic variants, especially in the case of rare genetic variants. In this case, it is not easy to conduct a translational clinical trial to validate the clinical relevance, because it is very hard to recruit those study subjects who have very rare genetic variants. In addition, the drug response of subjects will be much more complicated to predict the individualized precision dosing based on pharmacogenotypes if it is influenced by many combined factors, e.g. genetic variants + old age + drug interaction + renal dysfunction. All these issues are a big scientific hurdle to implement the genotype guided personalized medicine in the given individual patient.

The model informed approaches including physiologically-based pharmacokinetic (PBPK) model may be a potential tool to solve these issues in the implementation of pharmaco-genomics based precision medicine. The presentation will cover the concept and example of model based personalized precision medicine/ dosing of therapeutic drugs to predict the clinical PK and optimum individualized dose of an individual patients whose drug response is influenced by many genetic and non-genetic factors.

Keywords: Pharmacogenomics, clinical implementation, model informed precision dosing

Invited Speaker (Symposium IV)

Clinical Applications of PK/PD in Pediatric Antimicrobial Drug Dosing

Noppadol Wacharachaisurapol

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

E-mail: noppadol.w@chula.ac.th

Abstract

Pharmacokinetics (PK) and pharmacodynamics (PD) are crucial in optimizing antimicrobial drug dosing in pediatrics similar to adults. However, in pediatrics, PK parameters can vary significantly compared to adults due to factors such as differences in body size and composition, organ function, and drug metabolism. Moreover, even among the pediatric population, there are variations regarding ontogeny. In addition, pathophysiologic changes such as sepsis and augmented renal clearance can also affect PK parameters in such populations. These variations can lead to subtherapeutic or toxic levels. Inappropriate drug levels can then lead to unfavorable treatment outcomes. Apart from PK, PD is also vital for drug dosing. PD of antimicrobial agents refers to the relationship between the concentration of the drug in the body and the antimicrobial effect on the target microorganism. Currently, multidrug-resistant organisms with increased minimum inhibitory concentrations (MICs) are rising and compromising the PK/PD target achievement. Therefore, historical dose recommendations in package inserts might not be suitable for the current situation.

The application of PK/PD principles in pediatric antimicrobial drug dosing can improve treatment outcomes, reduce toxicity, and decrease the emergence of resistance. In addition, population PK modeling can be utilized to predict optimal dosing regimens for children based on their PK parameters. Furthermore, therapeutic drug monitoring (TDM) in line with a proper microbiological workup such as MIC could improve individualized antimicrobial drug dosing.

In conclusion, PK/PD principles are essential in pediatric antimicrobial drug dosing to optimize treatment outcomes and reduce the emergence of resistance. However, further research is needed to improve our understanding of PK parameters in children and to develop practical PK/PD-guided dosing strategies.

Keywords: Pharmacokinetics, Pediatrics, multidrug-resistant organisms, antimicrobials

Invited Speaker (Symposium IV)

Current Use and Promising Future of Gene Therapy in Thailand

Oranee Sanamaneechai

Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

E-mail: oranee141@gmail.com

Abstract

Since gene therapy's proof-of-concept was demonstrated as early as 1990. In 2017, US FDA approved voretigene neparvovec-rzyl for the treatment of patients with confirmed biallelic *RPE65* mutation-associated retinal dystrophy. In 2019, onasemnogene abeparvovec had been approved for the treatment of pediatric patients less than 2 years of age with spinal muscular atrophy (SMA). The author had experience of using gene therapy for SMA patient over the last 2 years and going to share the result of treatment.

Gene therapies introduce or modify genetic material, treating disease by editing or replacing the underlying genetic code. With gene therapy, new genes can be added, deleted, turned off or even replaced. Route of gene therapy can be various according to the organ of targeting to treat or prevent disease. Gene therapy is a technique that modifies a person's genes to treat or cure disease. Gene therapies can work by several mechanisms:

- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

Keywords: Gene therapy, spinal muscular atrophy, vector

Invited Speaker (Symposium IV)

Development of Sustainable Cellular Therapy for Blood Cancer in Thailand

Koramit Suppipat

Affiliation Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

E-mail: Koramit.S@chula.ac.th

Abstract

Chimeric Antigen Receptor (CAR) T-cell therapy has become a new standard of care for refractory and relapsed lymphoma patients. Viral vector-based CAR T-cells is considered the standard manufacturing method; however, high costs and complex manufacturing processes have limited the widespread use of CAR T-cell therapy. Currently, we are developing the state of the art of the transposon-based gene transfer using PiggyBac transposon system for CAR T immunotherapy, as a valid cost-effective and safe alternative to the viral vector-based systems. We recently completed the first cohort of phase I clinical trial on CD-19 PiggyBac CAR T cell in relapse and refractory lymphoma patient with good safety profile and promising treatment outcome.

Keywords: Chimeric antigen receptor T cell, PiggyBac transposon, lymphoma

A01

A Deep Learning-Based Approach for Identifying New Acetylcholinesterase Inhibitors from Mushroom Molecular Database for Alzheimer's Disease

Thana Sutthibutpong[1,2], Kewalin Posansee[2], Monrudee Liangruksa[3], Teerasit Termsaithong[4], Supanida Piyayotai[4], Paripok Phitsuwan[5], Patchreenart Saparpakorn[6], Supa Hannongbua[6], Teeraphan Laomettachit[7,*]

[1] *Center of Excellence in Theoretical and Computational Science (TaCS-CoE), Faculty of Science, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand*
[2] *Theoretical and Computational Physics Group, Department of Physics, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand*
[3] *National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand*
[4] *Learning Institute, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand*
[5] *Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand*
[6] *Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand.*
[7] *Bioinformatics and Systems Biology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand*

*E-mail: teeraphan.lao@kmutt.ac.th

Abstract

Alzheimer's disease (AD) is the most common type of dementia that gradually impairs a person's thinking and memory abilities to carry out even the most basic tasks and daily activities. Cholinesterase inhibitors (ChEIs) have been proven to alleviate AD symptoms and might even reduce the disease course, in which acetylcholinesterase inhibitors (AChEIs) represent a prospective area of AD drug development. However, the medication could have adverse side effects and cannot entirely halt the progression of the disease, limiting its efficacy. As a result, numerous attempts have been undertaken to search for alternative medicines with various structures and inhibitory actions using several computer techniques. A potential field of research is naturally derived-AChEIs because of fewer side effects on humans. In this study, candidates for AChEIs are found using a ligand-based virtual screening based on deep learning algorithms from a database that contains bioactive compounds from mushrooms (bacmushbase.sci.ku.ac.th). The evaluation metric, so called AUC (area under the curve), is predicted to be 0.98, suggesting the model's performance in identifying AChEI candidates. Following that, the top ten predicted compounds are further validated using docking and molecular dynamics (MD) simulations. Then the binding energies and modes of the AChE and the inhibitors are compared with that of the FDA-approved drug (donepezil). These methods can accelerate the development process by assisting with screening and directing the selection of new inhibitor candidates.

Keywords: Alzheimer's disease, deep learning, acetylcholinesterase inhibitors, natural products, molecular dynamics simulations

A02

Identification of Microtubule-Associated Proteins as Promising Biomarkers in Lung Cancer Based on TCGA Data Analysis

Natsaranyatron Singharajkomron[1], Varisa Pongrakhananon[1,2,*]

[1] *Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*
[2] *Preclinical Toxicity and Efficacy, Assessment of Medicines and Chemicals Research Unit, Chulalongkorn University, Bangkok, Thailand*

*E-mail: varisa.p@pharm.chula.ac.th

Abstract

Lung cancer has been the most common cause of cancer-related death**.** Most of the patients were diagnosed in an advanced state and displayed an aggressive progression. The lack of effective biomarkers is the main problem for lung cancer treatment. Therefore, the essential biomarkers for the diagnosis and treatment of lung cancer are urgently needed. Microtubule-associated proteins (MAPs) have been shown to govern several cellular processes. Interestingly, the expression level of MAPs was found to correlate with cancer progression. In this study, the diagnostic and prognostic biomarkers hinge on the differentially expressed MAP genes (DEMGs) were identified and validated across the Cancer Genome Atlas (TCGA) data, clinical samples, and Gene Expression Omnibus (GEO) data. The expression of 320 MAP genes, identified from the UniProt database, was compared between normal and tumor lung tissues using the data obtained from TCGA database which lung adenocarcinoma (LUAD) (56 normal and 451 tumor tissues) and lung squamous cell carcinoma (LUSC) (51 normal and 417 tumor tissues) subtypes were included. The prognostic significance was determined by Kaplan–Meier and Cox regression methods using the overall survival data. The expression profile of candidate genes was validated in patient lung tissues by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and in three independent datasets by GEO data analysis. Differential expression analysis demonstrated 47 upregulated and 41 downregulated genes. Survival analysis of the genes displayed high fold changes suggested 5 upregulated (*NUF2*, *KIF4A*, *KIF18B*, *DLGAP5*, and *NEK2*) genes and 1 downregulated (*LRRK2*) gene tightly associated with overall survival, providing potential molecules as lung cancer biomarkers. The six candidates can be used as diagnostic biomarkers in LUAD and LUSC. *NUF2*, *KIF4A*, *KIF18B*, *DLGAP5*, and *NEK2* for LUAD and *LRRK2* for LUSC can be used as prognostic biomarkers.

Keywords: Lung cancer, microtubule-associated protein, biomarker, overall survival

A03

The Antidiabetic Effect of Nidulin and Its Derivatives on Intracellular Glucose Uptake Stimulation

Tatchagrit Yosfungkul[1], Kanittha Chantarasakha[2], Masahiko Isaka[2], Surapun Tepaamorndech[3,*], Sutharinee Likitnukul[4,*]

[1] *Biomedical Science (International Program), Faculty of Science, Mahidol University, Bangkok, Thailand*
[2] *National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathum Thani, Thailand*
[3] *Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
[4] *Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*

*E-mail: sutharinee.lik@mahidol.edu, surapun.t@chula.ac.th

Abstract

Due to unhealthy eating habits and a sedentary lifestyle, the prevalence of type 2 diabetes mellitus (T2DM) has increased rapidly worldwide. Intracellular glucose uptake stimulation is known as an important target to prevent T2DM progression. The present study aimed to discover the novel compounds and their chemically modified derivatives containing the glucose uptake stimulating effect from fungal diversity in Thailand. Our results uncovered the secondary metabolite of *Aspergillus nidulans* known as nidulin and its semi-synthetic derivatives which is the chemical modification with a pyridine ring at the side chain called pyridylnidulin. Briefly, the cytotoxicity and glucose uptake activity of nidulin and pyridylnidulin at 5-100 μM were evaluated in 3T3-L1 adipocytes. The results indicated that nidulin promoted the 2-DG uptake activity in a dose-dependent manner at 5-50 μM with 151.97%, and enhanced glucose transporter 1 (GLUT1) and 4 (GLUT4) translocations into the cell membrane. Also, pyridylnidulin promoted the 2-DG uptake activity in a dose-dependent manner at 5-10 μM with 129.30%, which provided more potent glucose uptake stimulation when compared to nidulin at the same concentration. In summary, the present study exhibited the glucose uptake stimulating effect of nidulin and pyridylnidulin in a dose-dependent manner which should be interesting for T2DM drug discovery. A further study will be established to determine the pharmacological effect of both compounds in the T2DM animal model.

Keywords: Type 2 diabetes, glucose uptake, nidulin, pyridylnidulin

A04

Effects of Deferiprone on Oxidized Cholesteryl Esters in Low Density Lipoprotein and High Density Lipoprotein in β-Thalassemia

Pakawit Lerksaipheng[1], Kittiphong Paiboonsukwong[2], Pimtip Sanvarinda[1], Rataya Leuchapudiporn[3], Ken-Ichi Yamada[4], Noppawan Phumala Morales[1,*]

[1] *Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*
[2] *Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Salaya Campus,Nakhon Pathom, Thailand*
[3] *Department of Pharmacology and Physiology, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand*
[4] *Physical Chemistry for Life Science Laboratory, Faculty of Pharmaceutical Science, Kyushu University, Fukuoka, Japan*

*E-mail: noppawan.phu@mahidol.ac.th

Abstract

Oxidation of low-density and high-density lipoproteins (LDL and HDL) has been implicated in vascular complications in β-thalassemia. Our previous studies demonstrated that the core lipids, cholesteryl esters, were the major target of lipid peroxidation in lipoproteins. This study aims to evaluate the effects of a 2-week intermission of deferiprone, an iron chelator, on the changes of the oxidized cholesteryl esters (CEs), including cholesteryl linoleate (CE18:2) and cholesteryl arachidonate (CE20:4). LDL and HDL were separated from the serum of thirteen β-thalassemia patients and four healthy volunteers. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to analyze the lipids extracted from LDL and HDL. The results demonstrated the significantly higher concentration of CE-hydroperoxides (CE-OOH), but not their reduced form CE-OH, in LDL of thalassemia patients. On the other hand, both CE-OOH and CE-OH were significantly higher in the HDL of the patients. The production of oxidized CEs was pronounced after a 2-week intermission of deferiprone therapy, mainly oxidized CE18:2-OOH. Moreover, the decrease in the concentration ratio of CE-OH/CE-OOH suggested that the reaction preferentially moved towards oxidation rather than reduction. In conclusion, chelation therapy with deferiprone is a mainstay treatment in β-thalassemia. It delayed the lipid peroxidation chain reaction in the lipoproteins.

Keywords: β-Thalassemia, deferiprone, iron overload, oxidized lipoproteins, lipid hydroperoxides

A05

Effects of Thai *Mucuna pruriens* (L.) DC. Seed Extracts on Behavior and Motor Coordination in Alcohol-Treated Mice

Jirayut Kaewmor*, Sirinapa Rungruang, Kutcharin Phunikhom, Jintana Sattayasai, Charshawn Lahnwong

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

*E-mail: Jirayut.k@kkumail.com

Abstract

It is known that alcohol exposure causes disturbance of neuronal functions associated with oxidative stress and altered balance of neurotransmitters in the brain. *Mucuna pruriens* has long been used in traditional medicine for various neurological diseases as it has antioxidant property, neuroprotective and neurorestorative effects which help promote brain health. In the present study, we aimed to investigate the effects of Thai *Mucuna pruriens* (T-MP) seed aqueous extract on animals' behaviors and motor coordination during acute ethanol consumption in mice. Mice were randomly divided into 9 groups of 8 animals each and forced fed with either water or T-MP extract (300 or 600 mg/kg). Then one hour after, fed with either water or ethanol (2 or 6 g/kg). Thirty minutes after the second treatment, mice were subjected to the exploratory, elevated plus maze (EPM), tail suspension (TST), and rota-rod tests. the results were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test and $P < 0.05$ was considered as statistical significance. Ethanol-treated mice significantly increased anxiety-like behavior in the exploratory test, decreased time spent in open arms in the EPM test, increased immobility time in TST, and decreased time on the rod in the rota-rod test. Pre-treatment with T-MP, especially at 600 mg/kg, significantly alleviated the effects of alcohol in inducing abnormal behaviors and impairment of motor function in all tests. In conclusion, these data indicated that T-MP has a beneficial effect in controlling hyperactivity, impaired motor coordination, depressive-like and anxiety-like behavior induced by acute ethanol consumption in mice.

Keywords: *Mucuna pruriens*, alcohol, motor, behavior, mice

A06

Combination of PKI-402 and Infigratinib Induced G2/M Cell Cycle Arrest and Autophagic Cell Death through the FGFR and PI3K/mTOR Pathways in Cholangiocarcinoma Cells

Narumon Mahaamnard[1,2], Piman Pocasap[1], Auemduan Prawan[1,2], Laddawan Senggunprai[1,2], Veerapol Kukongviriyapan[1,2], Sarinya Kongpetch[1,2,*]

[1] *Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[2] *Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand*

*E-mail: sarinyako@kku.ac.th

Abstract

Cholangiocarcinoma (CCA) is a malignant tumor with a poor prognosis and unsatisfied treatment efficacy. Fibroblast growth factor receptor (FGFR) is one of targetable mutation in CCA. Recently, at least two FGFR inhibitors including infigratinib, have been approved by the US.FDA. to treat the metastatic CCA. However, many patients who initially respond to infigratinib develop resistance and relapse, with the activation of PI3K/mTOR pathway. Therefore, we aimed to investigate the effect of dual PI3K/mTOR inhibitors (PKI-402) combined with FGFR inhibitor (infigratinib), to suppress the oncogenic phenotypes in CCA cell lines. KKU-100 and KKU-M213A showed highly expressed FGFR2 and mTOR levels, were employed. The cytotoxicity of PKI-402 and infigratinib, was determined by Sulforhodamine B (SRB) Assay. The antiproliferation and apoptotic cell death, were determined by Acridine Orange/Ethidium Bromide (AO/EB) staining assay and AnnexinV/PI flow cytometry. Cell cycle arrest and autophagic cell death were determined by flow cytometry and AO staining assay. The protein expression of downstream effectors was determined by immunoblotting. The combination group of infigratinib and PKI-402 significantly induced antiproliferative effect through G2/M cell cycle arrest when compared to the single inhibitors. In addition, the combination group markedly induced apoptotic effect in CCA cells when compared to the single inhibitor. Moreover, the combined inhibitors also promoted CCA cell death through the induction of autophagy, as indicated by the upregulation of LC3B-II expression which is an important autophagic protein. Our study demonstrated that the combined inhibition of FGFR and PI3K/mTOR pathways potentiated the suppression of oncogenesis in CCA cells. Thus, this combination regimen could be a promising therapeutic strategy to be further evaluated in *vivo* models as well as in clinical studies. This work was financially supported by the granted by Faculty of Medicine, Khon Kean University, Thailand (grant no. IN65135) and the NSRF under the Basic Research Fund of Khon Kaen University through Cholangiocarcinoma Research. Narumon Mahaamnad was supported by postgraduate study support grant of faculty of medicine, Khon Kaen University.

Keywords: Cholangiocarcinoma, FGFR inhibitor, dual PI3K/mTOR inhibitor, FGFR alterations, drug resistance, autophagic cell death

A08

Effects of *Polycephalomyces nipponicus* Aqueous Extract on Motor and Neurobehavior in Acute Alcohol-Treated Mice

Sirinapa Rungruang[1,*], Jirayut Kaewmor[1], Kutcharin Phunikom[1], Jintana sattayasai[1], Charshawn Lanhwong[1], Araya Supawat[2]

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

*E-mail: Sirinapa.r@kkumail.com

Abstract

Acute alcohol intoxication is a clinically harmful condition that usually follows the ingestion of a large amount of alcohol. The acute central nervous system (CNS) effects of alcohol are result of increasing inhibitory GABAergic and reducing glutamatergic functions, leading to sedation, uninhibited behavior, impaired sensory and motor skills, and cognitive dysfunction. *Polycepholomyces nipponicus (P. nipponicus)* contains many active ingredients, especially adenosine. Adenosine can inhibit the release of glutamate by inhibiting Ca^{2+} channels which may help relieve the acute neurobehavioral effect of alcohol. However, the effect of this fungus on alcohol consumption has not been studied, so the aim of this study is to assess the effects of *P. nipponicus* extract on animal's motor and behavior in acute ethanol consumption. Mice were orally fed with either distilled water or *P. nipponicus* (200 or 600 mg/kg) extract, and one hour later, fed with either distilled water or ethanol (2 or 6 g/kg). Thirty minutes after the second treatment, motor and behavioral assays, including exploratory test, rotarod test, footprint analysis, and tail suspension test (TST) were performed Mice that received 6 g/kg of ethanol showed impaired motor functions as seen by a significant reduction in time on the rod in the rotarod test and a significant increase in both forelimb and hindlimb stride lengths when compared to normal control. The TST shows a significant increase in immobility time and anxiety-like hyperactive behavior was seen in exploratory test but mice that received 2 g/kg of ethanol showed the results same but not as good as the 6 g/kg group when compared to the control group. Oral treatment with *P. nipponicus* extract, especially at 600 mg/kg, could significantly reduce the acute effects of alcohol in all tests performed. In conclusion, the results of this study provide evidence that oral administration of *P. nipponicus* extract helps reduce the acute effects of alcohol on both motor functions and neurobehavior in mice. In at 200 mg/kg show could reduce the acute effects of alcohol in all tests performed but not significantly when compared to control group. Therefore, *P. nipponicus* may be one of the promising products to be used in treating the acute CNS effects of alcohol in the future.

Keyword: *Polycepholomyces nipponicus*, alcohol-treated mice, ethanol, motor, neuro-behavior

A09

Synthetic Lethality Based Therapy using Poly (ADP-ribose) Polymerase and Ataxia Telangiectasia Mutated Inhibitors in Cholangiocarcinoma Cells harboring DNA Damage Repair Gene Mutations

Rattanaporn Jaidee[1,2], Piman Pocasap[1,2], Veerapol Kukongviriyapan[1,2], Laddawan Senggunprai[1,2], Auemduan Prawan[1,2], Apinya Jusakul[1,3], Sarinya Kongpetch[1,2,*]

[1] *Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[2] *Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand*
[3] *Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*

*E-mail: sarinyako@kku.ac.th

Abstract

DNA damage response (DDR) mutations are one of the hallmarks contributing to the carcinogenesis of cholangiocarcinoma (CCA). Poly (ADP-ribose) polymerase (PARP) and ataxia-telangiectasia mutated (ATM) are the major proteins in the DDR pathway. DNA repair-deficient tumors are more sensitive to PARP inhibitors (PARPi) through the mechanism of synthetic lethality. Furthermore, DDR pathways mediated by ATM, are hypothesized to be essential survival pathways in response to PARPi. Therefore, this study aims to evaluate the antitumor effect of PARPi (olaparib, niraparib) and ATMi (AZD0156), on CCA cells harboring DDR mutations. Genomic alterations of DDR genes in CCA tumors were retrieved from the cBioPortal database. DDR gene mutations in CCA cell lines were assessed by whole exome sequencing. The relative cell viability and proliferation were assessed by MTS and colony formation assays. Cell death was determined using AO/EB staining and flow cytometry. Mutations were observed in 35.8% of cases from 684 CCA samples had alterations in at least one of four genes (*ARID1A*, *BAP1*, *BRCA2*, and *ATM*), suggesting their susceptibility to PARPi. High frequency of DDR gene mutations in 11 CCA cell lines showed the mutation of *BRCA2*, *CHEK1*, *FANCA*, and *ATR*. Of note, KKU-023 and KKU-100 (with *BRCA2*, *CHEK1*, *ATR*, and *ERBB2* mutation) showed the highest sensitivity to AZD0156 (IC_{50} 2.3 and 2.6 μM, respectively) while KKU-023 showed the highest sensitivity to olaparib (IC_{50} 6.3 μM) and niraparib (IC_{50} 4.3 μM). Moreover, the combination regimen of PARPi and ATMi showed a synergistic effect (combination index <1) on CCA cells to inhibit cell proliferation and promote cell apoptosis by 80% compared to the drug treatment alone which was less than 5% of apoptotic cells. Hence, combining PARPi and ATMi could be the potential therapy combating CCA harboring DDR mutations. This work was financially supported by the NSRF under the Basic Research Fund of Khon Kaen University through Cholangiocarcinoma Research and the Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program, Khon Kaen University (651H220).

Keywords: CCA, synthetic lethality, PARP inhibitor, ATM inhibitor, DDR mutation

A10

The Efficacy of Dapagliflozin in Improving Hemodynamic Status, Metabolic Alterations, and Vascular Function in High-Fat High-Fructose Diet-Induced Metabolic Syndrome Rats

Nattawut Chaisuk*, Patchareewan Pannangpetch, Kampeebhorn Boonloh, Panot Tangsucharit

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

*E-mail: nattawut_c@kkumail.com

Abstract

Metabolic syndrome (MS) is a common metabolic disorder that is a condition characterized by the existence of several risk factors for diabetes mellitus, nonalcoholic fatty liver disease and cardiovascular disease. Dapagliflozin (DAPA) is a sodium-glucose cotransporter-2 (SGLT2) inhibitor widely used to lower blood sugar levels in diabetic patients and has cardioprotective effects in congestive heart failure patients. However, few studies have investigated the underlying mechanism DAPA for vascular function. The aim of our study was to investigate the effect of DAPA on the hemodynamic status, alterations metabolic profile and mechanism of vascular function in high-fat high-fructose diet (HFFD) induced metabolic syndrome rats. Male Wistar rats were fed a normal or high-fat high-fructose diet for 10 weeks, and the metabolic syndrome animals were then treated with 1 and 5 mg/kg/day DAPA, 200 mg/kg/day metformin or no treatment for next 6 weeks. After the experiment, body weight, fasting blood glucose (FBG), oral glucose tolerance (OGT), serum insulin, lipid profile and hemodynamic parameters were measured. The vascular reactivity was measured by using mesenteric vascular bed technique. The metabolic syndrome rats showed significantly increased body weight, FBG, OGT, homeostasis model assessment of insulin resistance (HOMA-IR) scores, systolic blood pressure (SBP), mean arterial pressure (MAP), impaired lipid profile and vascular tone compared to normal control rats. DAPA significantly reduced body weight from 104.75±8.79 to -31.11±15.82 g, FBG from 115± 2.83 to 90.88±2.84 mg/dl, OGT from 19566±1447 to 15155±490 mg.min/dl of area under the curve (AUC) of blood glucose from 0-120 min, HOMA-IR from 3.32±0.45 to 0.9±0.09 scores, and systolic blood pressure from 128.96±5.80 to 88.37±2.46 mmHg relation to MS control group. Additionally, DAPA significantly improved lipid profile and vascular tone, with an upward trend of vasorelaxation and a downward trend of vasoconstriction on mesenteric arteries in relation to MS control group. In conclusion, our data provided evidence that DAPA appears to be an effective treatment for metabolic syndrome, as it alleviates diabetic phenomena and improves vascular function. Therefore, DAPA may be a potential treatment option for patients with metabolic syndrome to prevent the development of pre-diabetes and cardiovascular disease. Mr. Nattawut Chaisuk and this research were financially supported by a postgraduate scholarship, Faculty of Medicine, Khon Kean University, Thailand, and Khon Kaen University Faculty of Medicine invitation research grant.

Keywords: Dapagliflozin, SGLT2 inhibitor, metabolic syndrome, high-fat high-fructose diet, blood pressure, vascular function

A11

An Extract from Suk Sai-Yad on Monoamine Oxidase Inhibitory Actions and Depressive-Like Behavioral Tests in Mice

Wuttipong Masraksa[1], Orawan Monthakantirat[2], Charinya Khamphukdee[3], Supawadee Daodee[2], Yaowared Chulikit[2,*]

[1] *Graduate student, Program in Research and Development in Pharmaceuticals, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*
[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*
[3] *Department of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*

*E-mail: yaosum@kku.ac.th

Abstract

Introduction: Monoamine oxidases (MAOs) in humans have two isoforms: MAO-A and MAO-B, which are found in neurons and astroglia. Inhibiting these enzymes can increase neurotransmitter levels and relieve symptoms of depressive disorder. The Suk Sai-Yad (SSY) remedy was effective in treating sedative-hypnotic, analeptic, tonic, and panacea conditions from the forty-fourth traditional medicine recipe recorded in "The Royal Scripture of King Narai's Medicines". However, there have been no scientific reports of its inhibitory effect on the MAOs enzyme, which plays an important role in serotonin neurotransmitters. **Objective:** This study aimed to investigate the antidepressant-like effect of SSY on the inhibitory effect on MAOs and on behavioral models of depression, a forced swimming test (FST), and a tail suspension test (TST). **Materials and Methods:** *In vitro* studies using human recombinant MAO-A and MAO-B enzymes were performed for screening MAOIs in the SSY remedy. IC_{50} values were determined for these chimeric enzymes compared with MAO-selective substrates, clogyrine and deprenyl. For *in vivo* studies, mice were induced to depress by unpredictable chronic mild stress for 6 weeks. At week 3, the stress-mice were administered the SSY extract (20, 100, and 500 mg/kg/day, p.o.) and a reference antidepressant, imipramine (IMP, 20 mg/kg/day, p.o.) for 6 weeks. A behavioral test was started in week 5. The FST and TST were performed to evaluate depressive-like behavioral. **Results:** The SSY inhibited MAO-A and MAO-B activity with IC_{50} values of 49.72 ± 9.42 and 329.23 ± 50.80 µg/ml, respectively. Moreover, the SSY and IMP significantly decreased the immobility time compared with a control group in both TST and FST. **Conclusion:** The results of this study suggest that SSY ameliorates depressive-like symptoms, and the possible mechanism may be its inhibitory effect on MAOs enzymes.

Keywords: MAOs, MAOIs, depression, Suk Sai-Yad (SSY), depression-like behavior

A12

Developed and Validated LC-MS/MS Method to Determine Quetiapine and Metabolite Plasma Levels in Dementia

Sootthikarn Mungkhunthod[1,*], Santirhat Prommas[3], Apichaya Puangpetch[3], Pongsatorn Paholpak[2], Nontaya Nakkam[1], Chonlaphat Sukasem[3,4], Wichittra Tassaneeyakul[1], Suda Vannaprasahta[1]

[1] *Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[2] *Department of Psychiatry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
[3] *Division of Pharmacogenomics and Personalized Medicine, Departments of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[4] *Pharmacogenomics and Precision Medicine, Bumrungrad Genomic Medicine Institute (BGMI), Bumrungrad International Hospital, Bangkok, Thailand*

*E-mail: Sootthikarn_m@kkumail.com

Abstract

Background and Objective: Quetiapine (QTP) is a common atypical antipsychotic drug used worldwide to treat schizophrenia, acute mania, bipolar depression, and dementia. This study developed and fully validated to measure QTP and norquetiapine (NQTP) in human plasma using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method by US-FDA guidelines. **Method:** One-step liquid-liquid extraction is used a small plasma volume for extraction with acetonitrile. The analytes were separated on an XBridge BEH C18 column (3.0 x 50 mm, 2.5 µm). The mobile phase was 0.1% formic acid in 10 mM ammonium formate with 100% acetonitrile (pH 3.0). The chromatogram was performed in gradient mode at a flow rate of 0.3 ml/min, with a short analytical run time of 6 minutes. Multiple reaction monitoring (MRM) in positive ion mode was used to quantify QTP, NQTP, and risperidone as internal standards. **Results:** The calibration curve was 2.0-600.0 ng/ml linear range for QTP and NQTP in human plasma. The lower limit of detection for QTP and NQTP was 2 ng/ml. The coefficient of variation for intra-day and inter-day precision of QTP was within 3.6-11.2%, and accuracy was within 96.7-111.7%, whereas NQTP precision was within 5.3-12.9% and accuracy was within 93.2-110.5%. This method assessed the QTP and NQTP levels of 20 samples in dementia patients. The mean dose of QTP was 216.25 ± 284.04 mg/d (dose range 25-1100 mg/d). The mean concentrations of QTP and NQTP were 50.07 ± 53.68 and 29.25 ± 35.47 ng/ml, respectively. **Conclusions:** The validated LC-MS/MS method was successfully applied to evaluate the QTP and NQTP levels in dementia patients.

Keywords: Quetiapine, norquetiapine, atypical antipsychotic drug, LC-MS/MS, liquid-liquid extraction

A14

Synergistic Effect of *Stephania suberosa* Forman and Chlorhexidine Gluconate against Human Pathogenic Bacteria

Intu-orn Ayamuang[1], Yothin Teethaisong[2], Kittipot Sirichaiwetchakoon[3], Griangsak Eumkeb[3,*]

[1] *School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand*
[2] *Department of Medical Sciences, Faculty of Allied Health Sciences, Burapha University, Chon Buri, Thailand*
[3] *School of Preclinical Sciences, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand*

*E-mail: griang@g.sut.ac.th

Abstract

The rate of bacterial drug resistance has been increasing rapidly over the past few years. There are approximately 700,000 and 38,000 deaths annually from drug-resistant infections worldwide and in Thailand. The economic loss is estimated up to 3.5 trillion baht globally and 42 billion baht in Thailand. The synergy approach, combining two or more agents, has been recently emphasized to conquer this problem and to delay the development of antimicrobial resistance. *Stephania suberosa* Forman has traditionally been used to treat cancer, asthma, hyperglycaemia, inflammation, and bacterial infection for long time. However, the combination of *Stephania suberosa* extract (SSE) and chlorhexidine gluconate (CHG) against pathogenic bacteria has not been investigated. Hence, the objectives of this study were to investigate the synergistic activity of ethanol crude extract of SSE and 0.5% CHG in water (CHG 0.5W) and 2% CHG in alcohol (CHG 2A) against carbapenem-resistant *A. baumannii* (CRAB), *Pseudomonas aeruginosa* and *Streptococcus* spp. (*S. pyogenes*, and *S. mutants*) by the minimum inhibitory concentrations (MICs), checkerboard and time-killing curve determination. The results showed that MICs of CHGs (CHG 0.5W and 2A) and SSE against these strains were 128->2048 ug/ml and 0.063-0.5 mg/ml, respectively. The synergistic activities were observed in both CHG 0.5W+SSE and CHG 2A+SSE with an FIC index between 0.5 (synergism) in CRAB and 0.75-1 (partial synergism or addition) in *Pseudomonas aeruginosa* and *Streptococcus* spp. The time-killing assay confirmed the synergism activity of combinations killed CRAB from 6 h compared to CHGs alone. These findings support the use of the combination of SSE+CHG as an alternative antiseptic or disinfectant in hospital for slowdown bacterial resistant rate and decreased skin irritation when using antiseptic or disinfectant alone. For this reason, mode of action from synergistic activity should be further evaluated.

Keywords: Carbapenem-resistant *Acinetobacter baumannii*, chlorhexidine gluconate, plant extract, *Stephania suberosa* Forman, synergistic effect

A15

Development of a Physiologically Based Pharmacokinetic (PBPK) Model of Mescaline from Peyote Cactus (*Lophophora Williamsii*) in Rats and Humans

Manupat Lohitnavy[1,2,3,*], Kraivit Ngampasutadon[1,2], Thinnawat Kamchoom[1,2], Janthima Methaneethorn[1,2,3], Ornrat Lohitnavy[1,2,3]

[1] *Center of Excellence for Environmental Health & Toxicology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand*
[2] *Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand*
[3] *Pharmacokinetic Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand*

*E-mail: manupatl@gmail.com

Abstract

Mescaline is a psychedelic alkaloid found in peyote cactus (*Lophophora williamsii* and *Anhalonium lewinii*). Mescaline has been used it as a traditional medicine by Native Americans and is currently being developed as a treatment for psychological disorders. However, there have been reports of misuse and the safe dosage for its therapeutic use is unknown. This study aimed to develop a physiologically based pharmacokinetic (PBPK) model of mescaline to describe the concentration-time profiles of mescaline in rats and humans. A PBPK model structure consists of six organ compartments (i.e., lung, brain, liver, kidney, slowly, and rapidly perfused tissues). All the compartments were assumed to follow a flow-limited model, except the brain, which follows a diffusion-limited model. Parameters used in the model were derived from previous studies, calculations, or estimations based on available data from animals. A model validation was performed by comparing data from rats and humans. This developed PBPK model was able to predict mescaline concentration levels in the blood and brain of Wistar rats after a single subcutaneous injection of mescaline (20 mg/kg BW). Coefficient of determination (R^2) and mean absolute prediction error (MAPE) were 0.8515-0.9963 and 1.62-16.50%, respectively, In Sprague Dawley rats given a single Intraperitoneal injection of mescaline injection (40 mg/kg BW), The PBPK model could predict concentration-time profiles of mescaline in blood (R^2=0.72, MAPE=32.45%), brain (R^2=0.72, MAPE=18.69%), and liver (R^2=0.86, MAPE=29.09%). In addition, the model was able to predict mescaline blood concentration-time profiles in 5 human subjects following a single oral administration of mescaline (300 mg) with R^2 and MAPE ranging between 0.6088-0.9264 and 11.01-229.40%, respectively. In conclusion, the model could describe concentration-time profiles of mescaline in tissues of rats and humans. The developed PBPK model could the useful for predicting brain concentration levels and determining appropriate mescaline dosage regimens in humans.

Keywords: Physiologically based pharmacokinetic (PBPK) model, mescaline, peyote cactus, *Lophophora williamsii*, *Anhalonium lewinii*, psychoactive compound

A16

The Effect of Oral *Atractylodes lancea* (Thunb.) DC. Extract on Expression of Notch Signaling Pathway Genes

Ethan Vindvamara[1], Kesara Na-Bangchang[1,2], Wanna Chaijaroenkul[1,*]

[1] *Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand*
[2] *Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Thammasat University, Pathumthani, Thailand*

*E-mail: wn_ap39@yahoo.com

Abstract

Cholangiocarcinoma (CCA) is a cancer of the bile ducts and liver with a low survival rate. Development and progression of the disease involve many changes to gene expression, including genes involved in Notch signaling. The Notch signaling pathway is a highly conserved pathway that regulates differentiation, proliferation, and apoptosis among other things, mediated through direct cell-to-cell contact, and is implicated in many kinds of cancer including CCA. *Atractylodes lancea* (Thunb.) DC. (AL) is a plant used in traditional Thai medicine that has been shown to have anti-CCA activity. In this study, Notch pathway gene expression was analyzed from venous blood samples taken from Thai patients with advanced-stage CCA, participating in a phase II clinical trial. Patients were given either standard palliative treatment (control) or oral capsule pharmaceutical formulation of standardized crude ethanolic AL extract with 1,000-1,500 mg daily. In total, 15 patients were included in the study. Twenty-four genes were analyzed for differential gene expression by using nCounter® Analysis System, Nanostring Technology and data was analyzed by performing fold change analysis. Patients' gene expression on day 28 of treatment was compared against day 1 of treatment (baseline) for each treatment group. Data analysis revealed 20 genes had a significant fold change in expression from day 1 to day 28. Of these 20 genes, 7 genes had a significant fold change in expression in two treatment groups (DLL4, DTX4, HES1, HES5, HDAC1, APH1B, and LFNG), and 2 genes had a significant fold change in all three treatment groups (JAG1 and CREBBP). In conclusion, oral administration of standardized crude ethanolic AL extract affects the expression of Notch signaling pathway genes; most notably, JAG1 expression was down-regulated in response to this treatment, in a dose-dependent manner. Future research should observe how patient outcomes correlate to the expression of these genes.

Keywords: Cholangiocarcinoma, Notch signaling, *Atractylodes lancea*, differential gene expression

A17

Prevalence of the Artemisinin Resistance *Plasmodium falciparum* Kelch 13 Gene (*Pfk13*) in Malaria Patients from Thailand

Nutnicha Suphakhonchuwong, Jiraporn Kuesap*

Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand

*E-mail: jirajira28@yahoo.com

Abstract

To date, the drug resistance of *Plasmodium falciparum* is the major serious problem for malaria treatment worldwide. Dihydroartemisinin (DHA) and piperaquine (PPQ), the artemisinin-based combination therapies (ACTs), have been the first-line drug treatment for falciparum malaria in Thailand since 2016. Since *P. falciparum* Kelch 13 (*Pfk13*) has been established as a reliable molecular marker for surveillance of artemisinin and derivatives efficacy, the objective of this study was to investigate the prevalence of *Pfk13* in *P. falciparum*-infected patients after DHA/PPQ implementation (2016-2019) in Thailand. A total of one hundred *P. falciparum* isolates were collected from malaria patients in malaria endemic areas of Thailand. Nested polymerase chain reaction and sequencing were performed to detect the mutations in *Pfk13*. Of these, two different non-synonymous (16%) and one synonymous (1%) mutations were revealed. The presence of two novel non-synonymous mutations corresponding to partial artemisinin resistance were detected, including C580Y (13%) and R561Y (3%). Therefore, low frequency of *Pfk13* mutation suggested that the current drug regimen is probably suitable for treatment in this population. Continued surveillance of antimalarial drug resistance markers could be provided.

Keywords: *Plasmodium falciparum*, *P. falciparum* Kelch 13 (*Pfk13*), malaria

A18

High-Throughput Virtual Screening- and Molecular Docking-Based Prediction for AChE Inhibitors

Pitchayakarn Takomthong[1], Pornthip Waiwut[2], Carlo Ballatore[3], Kiattawee Choowongkomon[4], Chantana Boonyarat[1,5,*]

[1] *Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*
[2] *Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand*
[3] *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, California, United States*
[4] *Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand*
[5] *Center for Research and Development of Herbal Health Products, Khon Kaen University, Khon Kaen, Thailand*

*E-mail: chaboo@kku.ac.th

Abstract

Alzheimer's disease is the most common neurodegenerative disease, which has become a major social issue in recent years. This work aims to find new acetylcholinesterase (AChE) inhibitors, a target for Alzheimer's disease, by using computer-aided drug discovery approaches. Seven sets of ligands from the PubChem database were chosen for this investigation, including phenothiazines, phenoxazines, phenazines, acridones, acridines, xanthones, and carbazoles. A total of 17,330 ligands were submitted for high-throughput virtual screening to identify hits. Eight novel AChE inhibitors were identified, and then screened their physicochemical and pharmacological properties via SwissADME and pkCSM webserver. The outcome of in silico studies has suggested, all novel AChE inhibitors have shown promising drug-likeness, with better safety and efficacy profile for anti-Alzheimer's activity. Moreover, the hits have the potential to cross the blood-brain barrier (BBB), making them candidates for AD drug development studies. However, M5 belongs to carbazole and is the only ligand not showing hepatotoxicity. Therefore, further studies focusing on synthesis, in vitro pharmacological activities, and molecular dynamics simulations of M5 are required to confirm a better understanding of the efficacy, binding conformation of the identified compound.

Keywords: Virtual screening, Alzheimer's disease, acetylcholinesterase inhibitors, molecular docking, drug-likeness properties, BBB permeability, pharmacokinetic properties

A19

Effects of the Ethanol Extract from *Mesua ferrea* Linn. Flower on Biological Activities Related to Alzheimer's Disease

Kusawadee Plekratoke[1], Chantana Boonyarat[2], Orawan Monthakantirat[2], Natsajee Nualkaew[2], Jinda Wangboonskul[3], Suresh Awale[4], Yaowared Chulikhit[2], Supawadee Daodee[2], Charinya Khamphukdee[2], Suchada Chaiwiwatrakul[5], Porntip Waiwut[6,*]

[1] *Biomedical Science Program, Graduate School, Khon Kaen University, Khon Kaen, Thailand*
[2] *Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*
[3] *Faculty of Pharmaceutical Sciences, Thummasart University, Bangkok, Thailand*
[4] *Division of Natural Drug Discovery, Institute of Natural Medicine, University of Toyama, Toyama, Japan*
[5] *Department of English, Faculty of Humanities and Social Sciences, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, Thailand*
[6] *Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand*

E-mail: porntip.w@ubu.ac.th

Abstract

In searching for a promising candidate for the treatment of Alzheimer's disease (AD), the effects of ethanol extract of *Mesua ferrea* Linn. flower (MFE) on a pathological cascade of AD were determined by *in vitro*, and *in vivo* models. The results exhibited that the MFE extract showed antioxidant activity by both 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. The result from the Ellman method indicated that the extracts could inhibit acetylcholinesterase (AChE) function. In addition, MFE extract inhibited Aβ aggregation in thioflavin T assay and reduced human neuroblastoma cell (SH-SY5Y) death induced by H_2O_2 and Aβ. The result from western blotting analysis showed that the MFE extract inhibited H_2O_2-induced neuronal cell damage by downregulating the pro-apoptotic proteins, including Bax, cleaved caspase-3, and by increasing the expression of anti-apoptotic markers including MCl_1, BCl_{xl}, and survivin. Moreover, MFE extract enhanced the expression of neprilysin and inhibited the expression of APP, presenilin 1, and BACE. MFE extract also showed activity in an *in vivo* model by improving scopolamine-induced memory deficit in mice. The overall results exhibited that the MFE extract possesses a multimode of action involved with the AD pathology cascade including antioxidant, anti-AChE, anti-Aβ aggregation, and neuroprotection against oxidative stress and Aβ. Thus, the *M. ferrea* L. flower might be a potential candidate for further development as a drug for Alzheimer's disease.

Keywords: Antioxidant, acetylcholinesterase inhibition, beta-amyloid aggregation, neuro-protection

F01

Effects of *Centella asiatica* Extract (ECa 233) on Iron-Accumulated Brain in β-Thalassemic Mice

Puntita Anutagerngkun[1], Paranee Yatmark[2], Supachoke Mangmool[1], Pimtip Sanvarinda[1], Saovaros Svasti[3], Mayuree H. Tantisira[4], Noppawan Phumala Morales[1,*]

[1] *Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand*
[2] *Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand*
[3] *Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand*
[4] *Faculty of Pharmaceutical Sciences, Burapha University, Chonburi, Thailand*

*E-mail: noppawan.phu@mahidol.ac.th

Abstract

β-Thalassemia is among the most common inherited hemoglobin disorders found in Thailand. Due to their iron overload inducing oxidative stress, patients have manifested a variety of serious clinical presentations and complications, including neurodegenerative disorders, which cause cognitive dysfunction. A decreased expression of cellular iron export-related protein may increase brain iron accumulation and impair brain tissue and function. *Centella asiatica* extract has been widely known for its neuroprotective effects, besides its anti-inflammatory and antioxidant properties. The present study aims to investigate the mechanism of *C. asiatica* extract (ECa 233) on iron-accumulated brain involving the expression of ferroportin (FPN) and neuropathology in β-thalassemic (BKO) mice. The animal was induced to have an iron overload by the total injection of 100 mg of iron dextran followed by 200 mg of ECa 233 treatment in order to observe iron levels, histopathological changes, and FPN expression in the brain. The result showed that ECa 233 treatment upregulated the FPN protein with slightly a decreased brain iron level. In addition, the improvement of brain lesions was detected. This result suggested that ECa 233 may have the neuroprotective effect on iron-accumulated brain in the thalassemic mouse model.

Keywords: *Centella asiatica*, ECa 233, brain iron overload, neurodegeneration, thalassemia

Introduction

β-Thalassemia is among the most common inherited hemoglobin disorders found worldwide, including Thailand.[1,2] The mutation of hemoglobin genes can result in several severity degrees of clinical manifestation, including iron overload, which causes the majority of morbidity and mortality in thalassemia patients.[3] An excess accumulation of iron in vital organs due to either life-long blood transfusion or ineffective erythropoiesis generates reactive oxygen species (ROS) and induces oxidative stress, leading to a variety of complications, especially the dysfunction of the liver, heart, and endocrine glands.[2] Thalassemia patients can develop neurodegenerative complications, contributing to a cognitive impairment and a poor performance of motor functions.[4] Despite the fact that the mechanism of neurodegeneration remains unclear, the excess of iron deposit in the brain is considered one of the important

factors that cause the pathogenesis of complications.[5] A deregulation of iron transport-related proteins, e.g. a decreased activity of ferroportin (FPN), an increased activity of transferrin receptor 1 (TfR1), and divalent metal transporter 1 (DMT1), as well as hepcidin manipulation have been reported that may increase brain tissue iron levels and induce neuronal death.[5-7]

Centella asiatica (L.) Urban (Gotu kola) is a plant from the Apiaceae family that is commonly found in tropical areas, including Southeast Asia.[8] Since ancient times, the plant has been used as a traditional therapy for various conditions.[9] The *in vitro* and *in vivo* studies have demonstrated that the majority of active compound in the plant; asiatic acid, asiaticoside, madecassic acid, and madecassoside, have many pharmacological effects, such as anti-inflammatory, anti-oxidant, and neuroprotective effects.[9-11] Besides therapeutic effects, a standardized extract of *C. asiatica* (ECa 233) has been proven in rodents and humans that have a safety profile for use.[11,12] However, there were no reports about the effect of ECa 233 on neurodegeneration associated with brain iron overload in β-thalassemic mice. Therefore, this study aims to investigate the mechanism of ECa 233 on iron-accumulated brain involving the expression of FPN and neuropathology in β-thalassemic mice. The result from this study may clarify another neuroprotective effect of ECa 233. Additionally, this extract may be a promising alternative compound for relieving thalassemia complications in patients as well as improving their quality of life.

Methods

Drugs and reagents

ECa 233, which consists of triterprenoids glycosides not less than 80% and the ratio between madecassoside and asiaticoside was kept at 1.5 ± 0.5:1, was prepared by using a well-defined procedure (patent pending). The solution of ECa 233 was suspended in distilled water. Iron dextran was obtained from Sigma-Aldrich (Saint Louis, MO, USA).

Animals

The 12-month-old β-globin knockout mice (BKO) were bred at the Laboratory Animal Unit, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhon Pathom, Thailand. Three to five mice from the same groups were kept together in polystyrene cages at the room temperature of 22±2 ºC and the humidity of 55±15% under 12/12 h light/dark cycle. They were fed with regular chow and ultra-filtrated (UF) water *ad libitum*. All procedures in this study were approved by the Institute of Molecular Biosciences Animal Care and Use Committee, Mahidol University (Protocol No. 2020/014).

Experimental design

BKO mice were randomly divided into 4 groups (n=4-5 per group) and treated differently as follows: Control (BN), receiving only normal saline (0.2 ml/mouse) daily for 5 days; Iron overload (BI), receiving iron dextran (20 mg iron/mouse/day) for 5 days, following by normal saline (0.2 ml/mouse) daily for 10 days; Control ECa 233 (BE), receiving normal saline (0.2 ml/mouse) daily for 5 days, following by ECa 233 (20 mg/kg/day) for 10 days; Iron overload with ECa 233, receiving iron dextran (20 mg iron/mouse/day) for 5 days, following by ECa 233 (20 mg/kg/day) for 10 days. All treatments were given by intraperitoneal (i.p.) injection. At the end of the experiment, mice were euthanized with an overdose (50-60 mg/kg) of i.p. injection of Nembutal®. The brain in each group was quickly collected and fixed in 4% paraformaldehyde solution at the room temperature (n=1-2) or kept at -80 ºC (n=3) for later analyses.

Brain tissue iron content

Iron contents in the whole brain tissues were determined using a modified ferrozine-based colorimetric assay reported by Foy et al.[13]

Histopathological analysis

In brief, all fixed brains were embedded in paraffin and cut into 4 µm sections by using a microtome. The tissue sections were deparaffinized, dehydrated, and stained with hematoxylin and eosin (H & E) for histopathological change or with Perls' Prussian blue for detection of brain iron accumulation.

Measurement of iron transport-related proteins in brain tissues by Western blot method

FPN were measured by Western blot method. Briefly, the whole brain tissues were homogenated with RIPA buffer mixed with protease inhibitor cocktail. Proteins (25 µg) were separated by 10% SDS-PAGE gel and then transferred to PVDF membranes. Membranes were blocked with 5% bovine serum albumin in TBS-T solution. The interested protein was detected using the anti-ferroportin/SLC40A1 antibody (ab58695, Abcam).

Statistical analysis

Data was shown as median and range analyzing by IBM SPSS Statistics (version 21.0) software. Kruskal-Wallis test was used for determination of statistically significant differences between groups. Statistical significance is considered at $p < 0.05$.

Results

Brain tissue iron content

The result of the modified ferrozine-based colorimetric assay indicated the changes in total iron levels in the brain, as shown in **Figure 1**. The total loading of 100 mg of iron significantly increased the concentration of brain iron in the BI mice ($p < 0.05$), compared with that in the control groups. However, compared with the iron-loaded mice, the brain iron levels in the BIE groups were unchanged after ECa 233 treatment.

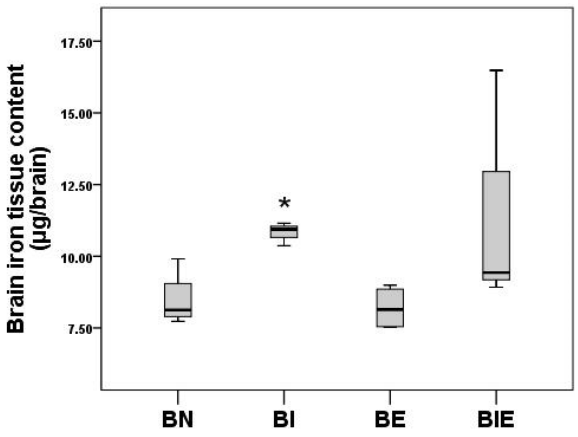


Figure 1. Effect of ECa 233 treatment on brain iron content in BKO mice. Values are median and range (n=3). *Significant difference from the control group ($p < 0.05$).

Brain histopathological change

Perls's Prussian blue staining was used to observe iron accumulation in brain tissue. The blue positive area indicates the tissue iron deposit. In the control groups, the Prussian blue positive areas were detected in the cerebral cortex and lateral ventricles at the caudal diencephalon. The spontaneous iron deposits were rarely observed in cerebral parenchyma and capillaries (**Figure 2**, 1A and 1B). After loading, multiple areas of iron accumulation were clearly increased, especially in the lateral ventricles, cerebral parenchyma and capillaries (**Figure 2**, 2A and 2B). In the ECa 233 treatment groups, the Prussian blue positive areas were reduced compared with the iron overload groups (**Figure 2**, 4A and 4B).

H & E staining was used to observe brain tissue histopathology. At the cerebral cortex (**Figure 2**, column C), iron overload caused significantly more brain damage (**Figure 2**, 2C), compared with that in the control mice. Multifocal of degenerating neurons, death cells, and vacuolization were distinctly observed. The treatment with ECa 233 gradually decreased neurodegeneration and ameliorated cerebral tissues (**Figure 2**, 4C). At the hippocampal region (**Figure 2**, column D), an increase of dark neurons, ghost cells, and vacuolization were found in the tissues of BI mice (**Figure 2**, 2D). In iron overload mice that received ECa 233 injections, those lesions were slightly improved (**Figure 2**, 4D), compared with the BI groups.

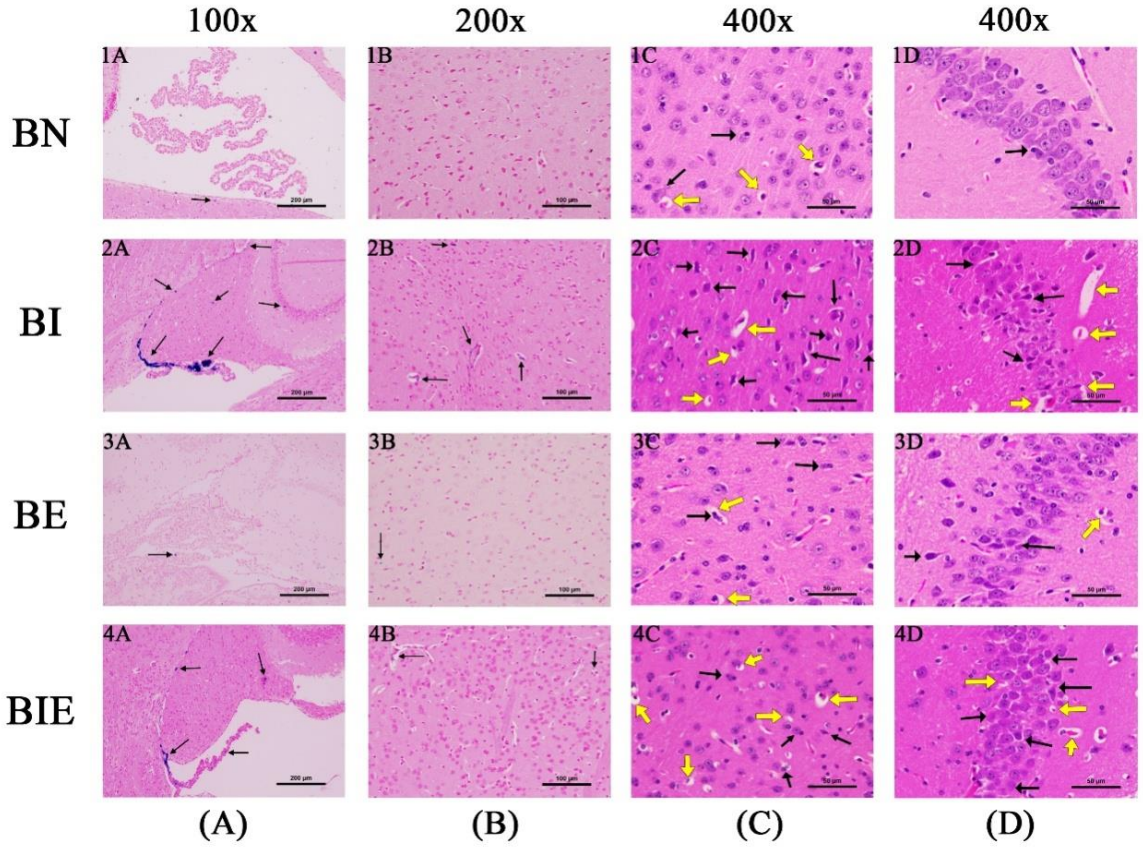


Figure 2. Effect of ECa 233 treatment on iron accumulation (column A-B) and histopathological changes (column C-D) in brain tissues of BKO mice. (Column A and B) The blue positive areas (black arrow) indicated the tissue iron deposit at the lateral ventricle (A, magnification 100x), and at the cerebral cortex (B, magnification 200x). (Column C and D) The H&E staining showed that, besides, neuron degeneration and neuronal death (black arrow), vacuolization (yellow arrow) were observed at cerebral cortex (C, magnification 400x), and at the hippocampal region (D, magnification 400x).

The expression of ferroportin in brain

Western blot analysis was used to examine the expression levels of the iron export-related protein, FPN, in the whole brain. The results were shown in **Figure 3**. In the BI mice, the levels of FPN expression were obviously decreased ($p < 0.05$), compared with that in control groups, which might produce the increased levels of brain iron tissues. After treatment with 200 mg of ECa 233, the expression levels of FPN in BIE mice were significantly upregulated ($p < 0.05$). Interestingly, the increased expression levels of this iron exporter were observed in mice treated with ECa 233 alone ($p < 0.05$), compared with that in the BN mice which were untreated. The significant difference of FPN expression levels between BN and BIE; BE and BIE was not observed.

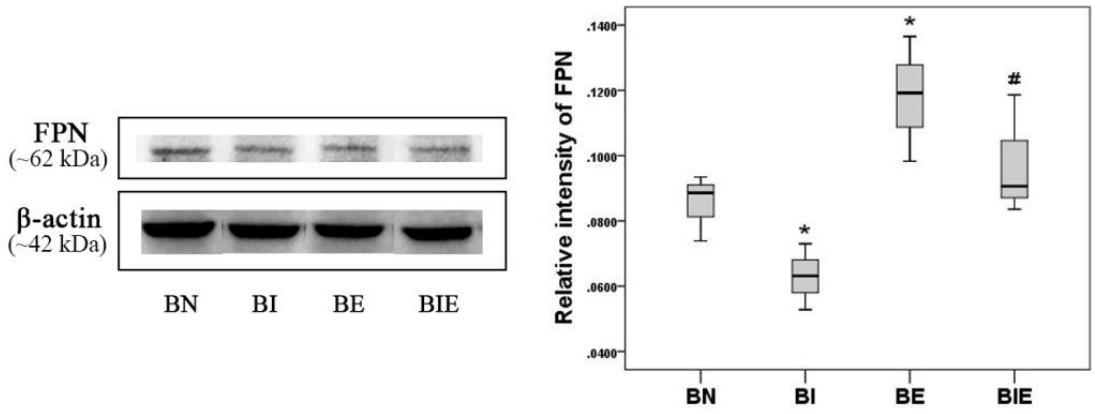


Figure 3. Effect of ECa 233 treatment on the expression of ferroportinin BKO mice. Values are median and range (n=3). *Significant difference from the control group ($p < 0.05$); #Significant difference from the iron overload group ($p < 0.05$).

Discussion

C. asiatica is a traditional herb that has been known for its high therapeutic value. The plant and its extract have been used for ages as a medicine in many countries and cultures.[14] Besides its anti-inflammatory and anti-oxidant properties, *C. asiatica* and its extract, including ECa 233 have been widely known as a "brain tonic" agent due to the protective effect of its bioactive compounds on neurodegenerative disorders such as Alzheimer's disease.[9,15,16]

The previous study has suggested that the excessive iron deposit in the brain under iron overload condition in thalassemia patients may cause cognitive impairment since cellular labile iron could produce ROS and induce oxidative stress, resulting in neuronal death.[4,6] Thus, this means that a decreased iron brain level may partly protect against neurodegeneration and improve cognitive function, in addition to other commonly known mechanisms, e.g. an increased anti-oxidant and anti-inflammatory activity. An increased expression of FPN, a cellular iron exporter, and an improvement in iron metabolism, e.g. a decreased expression of iron-uptake protein and a lower level of hepcidin in the brain may be another useful target for reducing iron deposit.

The present study demonstrated that an administration of iron to induce iron overload in thalassemic mice obviously decreased the expression of FPN and increased brain iron accumulation, leading to a serious brain injury. After treatment with ECa 233, the upregulation of FPN in the brain was definitely observed, even if the level of brain iron and neuro-degeneration were slightly reduced. It was suggested that ECa 233 may increase the cellular

iron export by inducing the expression of FPN and improving iron metabolism in the brain. This action may partly involve the neuroprotective mechanism of ECa 233.

Although the present study may not show a significant improvement in neuro-degeneration related to levels of brain iron accumulation due to a limitation of number of samples, this is the first investigation to reveal another possible effective mechanism of ECa 233 associated with its neuroprotective effect during iron overload. However, further studies with adequate numbers of total samples to confirm the effect of ECa 233 on brain iron metabolism involving the iron associated proteins (e.g. TfR1 and DMT1) and the related mechanisms (e.g. anti-oxidant and anti-inflammatory) against brain injury during iron overload need to be investigated. The investigation of hepcidin may be included in the study.

Conclusion

In summary, this study revealed the possibility for ECa 233 exerting protective effects on neurodegeneration associated with iron overload in the brain of thalassemic mice. Besides anti-oxidant and anti-inflammatory properties, the underlying mechanism at least partly involved decreasing brain iron accumulation through regulating an iron export-related protein, including FPN, by decreasing its expression. ECa 233 may become an additional treatment for the management of iron overload and complications in thalassemia patients.

Acknowledgement

This study was financially supported by Mahidol University (Fundamental Fund: fiscal year 2023 by National Science Research and Innovation Fund (NSRF), FF-128/2566).

References

1. Department of Medical Services MoPH, Thailand. Guidelines for the care of thalassemia patients in general practice. 2017.
2. Taher AT, Musallam KM, Cappellini MD. beta-Thalassemias. N Engl J Med 2021;384(8): 727-43.
3. Taher AT, Saliba AN. Iron overload in thalassemia: different organs at different rates. Hematology 2017;2017(1):265-71.
4. Duman O, Arayici S, Fettahoglu C, Eryilmaz N, Ozkaynak S, Yesilipek A, et al. Neuro-cognitive function in patients with β-thalassemia major. Pediatr Int 2011;53(4):519-23.
5. Bagwe-Parab S, Kaur G. Molecular targets and therapeutic interventions for iron induced neurodegeneration. Brain Res Bull 2020;156:1-9.
6. Origa R. beta-Thalassemia. Genet Med 2017;19(6):609-19.
7. Vela D. The dual role of hepcidin in brain iron load and inflammation. Front Neurosci 2018;12:740.
8. Chandrika UG, Prasad Kumarab PA. Gotu Kola (*Centella asiatica*): Nutritional properties and plausible health benefits. Adv Food Nutr Res 2015;76:125-57.
9. Belwal T, Andola HC, Atanassova MS, Joshi B, Suyal R, Thakur S, et al. Gotu Kola (*Centella asiatica*). In: Nabavi SM, Silva AS, editors. Nonvitamin and nonmineral nutritional supplements. 1st ed. London: Academic Press; 2019. p. 265-75.
10. Jew SS, Yoo CH, Lim DY, Kim H, Mook-Jung I, Jung MW, et al. Structure-activity relationship study of asiatic acid derivatives against beta amyloid (A beta)-induced neurotoxicity. Bioorg Med Chem Lett 2000;10(2):119-21.

11. Songvut P, Chariyavilaskul P, Tantisira MH, Khemawoot P. Safety and pharmacokinetics of standardized extract of *Centella asiatica* (ECa 233) capsules in healthy thai volunteers: a phase 1 clinical study. Planta Med 2019;85(6):483-90.
12. Chivapat S, Tantisira M. Acute and sub-chronic toxicity of a standardized extract of *Centella asiatica* ECa 233. Thai J Pharm Sci 2011;35:55-64.
13. Foy AL, Williams HL, Cortell S, Conrad ME. A modified procedure for the determination of nonheme iron in tissue. Anal Biochem 1967;18(3):559-63.
14. Lv J, Sharma A, Zhang T, Wu Y, Ding X. Pharmacological review on asiatic acid and its derivatives: a potential compound. SLAS Technol 2018;23(2):111-27.
15. Kunjumon R, Johnson AJ, Baby S. *Centella asiatica*: Secondary metabolites, biological activities and biomass sources. Phytomed Plus 2022;2(1).
16. Wanakhachornkrai O, Pongrakhananon V, Chunhacha P, Wanasuntronwong A, Vattanajun A, Tantisira B, et al. Neuritogenic effect of standardized extract of *Centella asiatica* ECa233 on human neuroblastoma cells. BMC Complement Altern Med 2013;13:204.

F02

Determination of *In Vitro* Antioxidant and Tyrosinase Activity of Ethanolic *C. indica* Inflorescences and Leaf Extract

Pakawadee Mahaprom, Pansakorn Tanratana*

Department of Pharmacology, Faculty of Science, Mahidol University, Thailand

*E-mail: pansakorn.tan@mahidol.ac.th

Abstract

Melanin is a pigment that is present in many living organisms, including humans. Melanogenesis is a process of melanin production, which requires the enzyme tyrosinase. Therefore, manipulation of this particular enzyme can affect the melanin production. Several plants have gained attention as a natural source of tyrosinase inhibitor due to the bioactive functions of phytochemical constituents such as phenolic compounds, as these compounds have been reported as one of the antioxidant molecules that can reduce the risk of oxidative stress-related diseases. *C. indica*, known as marijuana, has become one of the interesting plants due to their therapeutic effects of phytochemical constituents such as phyto-cannabinoids, and phenolic compounds. In this experiment, antioxidative compounds such as phenolic and flavonoid were measured in the water-soluble ethanolic extract of *C. indica* inflorescence and leaf. DPPH scavenging activity and tyrosinase activity were also investigated. The data showed that the amount of total phenolic contents in the inflorescence and leaf extract of *C. indica* was 0.0507 ± 0.034 mg GAE/mg, while the amount of flavonoid content was 0.0168 ± 0.024 mg QUE/mg. IC_{50} of DPPH scavenging activity was 0.137 ± 1.46 mg/mL, and *C. indica* expressed a dose-dependent anti-tyrosinase activity. However, further experiments are needed in order to test the cytotoxicity, and the effect of the extract at cellular levels.

Keywords: Tyrosinase, antioxidant, *Cannabis indica*, marijuana, melanogenesis

Introduction

Melanin is a pigment that is present in many living organisms, including humans. It is responsible for the coloration of the skin, hair, and eyes, as well as other parts of the body, and it also protects the skin from the damage of UV radiation.[1] Melanogenesis is a process of melanin production, which requires the enzyme tyrosinase. Therefore, manipulation of this particular enzyme can affect the melanin production.[2] The process of melanogenesis involves several enzymatic and chemical reactions that take place in specialized cells called melanocytes, which are primarily located in the skin, hair follicles, inner ear, bones, heart, and brain.[1] Several plants have gained attention as a natural source of tyrosinase inhibitor due to the phytochemical compounds such as polyphenols, as these compounds have been reported as one of the antioxidant molecules that can reduce the risk of oxidative stress-related diseases.[3] Marijuana (*Cannabis indica*), famous for its psychoactive effect, is the plant originally found in India and Persia. There are many groups of phytochemical constituents that exist in *C. indica*, the specific group is phytocannabinoids. Phytocannabinoids have gained a

lot of attention due to their wide range of medical benefits. The most well-known phyto-cannabinoids are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), the other phyto-cannabinoids such as cannabinol, cannabidivarin, cannabigerol, and cannabichromene also have been studied for their therapeutic effects.[4] These compounds react with special signaling systems that are found throughout the body called endocannabinoid system, it regulating the physiological function of the body.[5] Among all phytocannabinoids, THC has a psychoactive effect while other phytocannabinoids do not. The studies of phytocannabinoids revealed that they potentially can be used as drugs for anti-inflammatory, anti-tumor, and anti-obesity.[6] Other phytochemical compounds such as polyphenols, flavonoids, and terpenes in *C. indica* had been investigated[7,8], and reported to have therapeutic effects including antioxidants, anti-inflammatory, anti-diabetic, and anti-tyrosinase properties.[9-11] Recent findings reported that CBD increased melanogenesis in the melanocytes through the upregulation of tyrosinase-related protein 1 (TRP-1) and, tyrosinase-related protein 2 (TRP-2).[12] TRP-1 and TRP-2 are a group of enzymes responsible for melanin production in melanosomes, an increase of these enzymes results in more pigmentation on the skin.[13] This finding suggests that CBD could be an optional treatment for hypopigmentation disorders. However, the investigation of *C. indica* extracts on melanogenesis has not been reported.

Therefore, this present experiment is designed to evaluate the antioxidative activity of ethanolic extracts of *C. indica* in water-soluble fraction obtained from inflorescence and leaf and its effect on tyrosinase enzyme to provide additional scientific data for further development as a natural source of antioxidants and skin treatment.

Methods

Plant Material and Extraction

C. indica inflorescence and leaves were collected from Phusangtong farm, Chaiyaphum, Thailand. Dried inflorescence and leaves (26 g) were powdered and extracted with 500 mL of 70% ethanol for 3 days by sonicator bath at 55 ºC with a frequency of 80 kHz. The extract was filtered and dried by the rotary evaporator with 50 ºC of bath temperature.[14]

Total Phenolic Content (TPC)

The total phenolic content was determined by the Folin–Ciocalteu method with modifications.[15] Briefly, 30 µL of gallic acid (reference), or extracts were added with 2,370 µL of distilled water, then mixed with 150 µL of 10% (w/v) Folin-Ciocalteu reagent. The solution was mixed and incubated for 4 mins. Then, 450 µL of saturated (w/v) Na_2CO_3 was added. The sample was then mixed and incubated in the dark at room temperature for 40 min. All photometric measurements were performed by UV-VIS Spectrophotometer (Jasco V530) at 765 nm. The results were expressed as gallic acid equivalent (GAE) mg/mg extract.

Flavonoid Content (FC)

The flavonoid content was measured by colorimetric method[15] with modifications using aluminum chloride to react with quercetin and create a stable, color-signature complex. Briefly, 100 µL of quercetin standard solutions (reference), or extracts were added with 1,650 µL distilled water, and then mixed with 90 µL potassium acetate. Subsequently, 60 µL of 10% aluminum chloride ($AlCl_3$) and 900 µL of 95% ethanol were added. The samples were incubated in the dark at room temperature for 30 min. All measurements were measured by UV-VIS Spectrophotometer (Jasco V530) at 415 nm. The results were expressed as quercetin equivalent (QUE) mg/mg extract.

DPPH Free Radical Scavenging assay

The antioxidative activity of ethanol extract *C. indica* extracts was determined by the 2,2-diphenyl-picrylhydrazyl (DPPH) assay with modifications.[16] Briefly, 120 µL of trolox (positive control) or water-soluble fraction extracts in various concentrations were added with 1,080 µL of DPPH solution (whereas PBS was added instead of trolox or extracts as a control), then shaken and incubated in the dark at room temperature for 15 sec. All the measurements were measured by UV-VIS Spectrophotometer (Shimadzu) at 515 nm. The % inhibition was calculated using the following equation.

$$\%Inhibition = \frac{Absorbance_{Control} - Absorbance_{Sample}}{Absorbance_{Control}} \times 100\%$$

Tyrosinase activity

Mushroom tyrosinase activity was determined by the rate of oxidation of L-DOPA. The assay was performed according to the reported method[17] with modifications. Briefly, 30 µL of kojic acid (positive control) or water-soluble fraction of the extracts in various concentrations (whereas the PBS was added instead of kojic acid or extracts as a control), 70 µL of PBS, 50 µL tyrosinase enzyme (50 units/mL), and 50 µL of L-DOPA (1 mg/mL) were mixed. The plates were subsequently incubated at 37 ℃ for 10 mins, and the reaction was measured by a microplate reader (VarioskanFlash) at 492 nm. The % inhibition was calculated using the following equation.

$$\%Inhibition = \frac{Absorbance_{Control} - Absorbance_{Sample}}{Absorbance_{Control}} \times 100\%$$

Statistical analysis

All experiments were repeated at least two replicates. In vitro studies of phenolic and flavonoid contents, antioxidative activities, and tyrosinase inhibitory activities were presented as mean±SD. The statistical analysis was determined by using One-way ANOVA (GraphPad Prism program version 9). *P*-value less than 0.05 was considered statistically significant.

Results and discussions

The results demonstrated that the water-soluble ethanolic extract contained total phenolic content at 0.0507 ± 0.034 mg GAE/mg, while the flavonoid content was 0.0168 ± 0.024 mg QUE/mg **(Table 1)**. The free radicals scavenging activity as measured by the DPPH assay appeared to follow a dose-dependent manner. The IC_{50} of trolox was 0.0735 ± 1.93 mg/mL, while the IC_{50} of the ethanolic extract was 0.137 ± 1.46 mg/mL **(Figure 1)**. This result demonstrated that the ethanolic extract of *C. indica* inflorescence and leaf was approximately 50% less potent when compared with trolox (as a positive control). Antioxidative properties including the presence of total phenolic compounds, flavonoid compounds, and DPPH scavenging activity in the ethanolic extract of *C. indica* in water soluble fraction were compared to the previous studies. Results obtained from *C. indica* leaf extract by the microwave extraction methods using water solution contained total phenolic contents at 218.9 ± 0.9 mg GAE/g, the flavonoid contents at 88.6 ± 3.4 mg QUE/g and DPPH inhibition were 39.3 ± 6.1%. On the other hand, the maceration extraction method using methanolic solution contained total phenolic contents at 17.7 ± 1.1 mg GAE/g, the flavonoid contents at 10.5 ± 1.9 mg GAE/g, and DPPH inhibition was 38.0 ± 0.5 %.[18]

In addition, *C. indica* inflorescence and leaf extract dose-dependently exhibited anti-tyrosinase activity **(Figure 2B)**. Importantly, the anti-tyrosinase activity of the water-soluble fraction ethanolic *C. indica* inflorescence and leaf extract contradicted with an increased ability in melanogenesis of CBD.[12] The observed phenomenon could be due to the contributory

effect of phytochemical compounds such as phenolic compounds and flavonoids, which can also inhibit tyrosinase activity, found in leaves of this plant. In addition, other unidentified bioactive compounds from the extract may have had the anti-tyrosinase effect. Moreover, the pro-melanogenesis effects of CBD at cellular levels were observed in a culture of melanocytes.[12] Therefore, it cannot be concluded that the whole ethanolic extract of *C. indica* could reduce melanin contents in melanocytes from the in vitro results, necessitating further studies to observe its effects at the cellular level.

Table 1. Total phenolic and flavonoid content were expressed as the mean ± SD of two replicates from ten independent experiments. The value of total phenolic content was demonstrated as amounts of gallic acid equivalent (GAE) per amount of crude extract, and the flavonoid content was shown as amount of quercetin equivalent (QUE) per amount of crude extract.

Extract	Solvent	Total phenolic content (mg GAE/mg)	Flavonoid content (mg QUE/mg)
Inflorescence and Leaf	70% ethanol	0.0507 ± 0.034	0.0168 ± 0.024

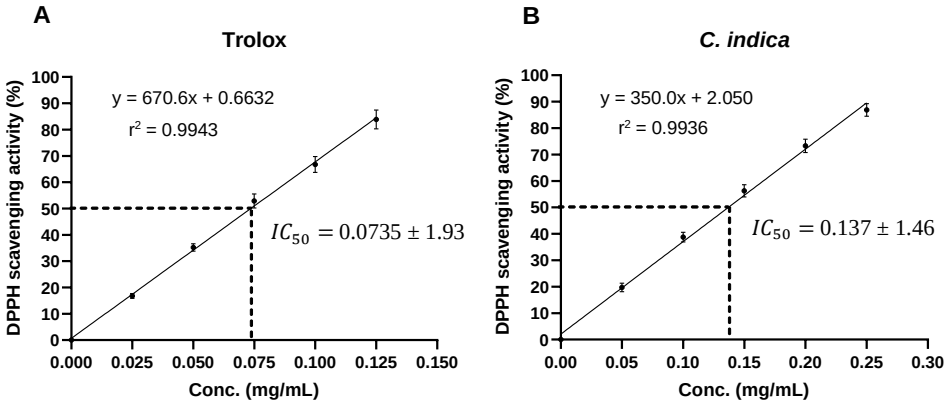


Figure 1. DPPH radical scavenging activity of trolox (A) and *C. indica* inflorescence and leaf extract (B). The results were expressed as the mean ± SD of two replicates from three independent experiments.

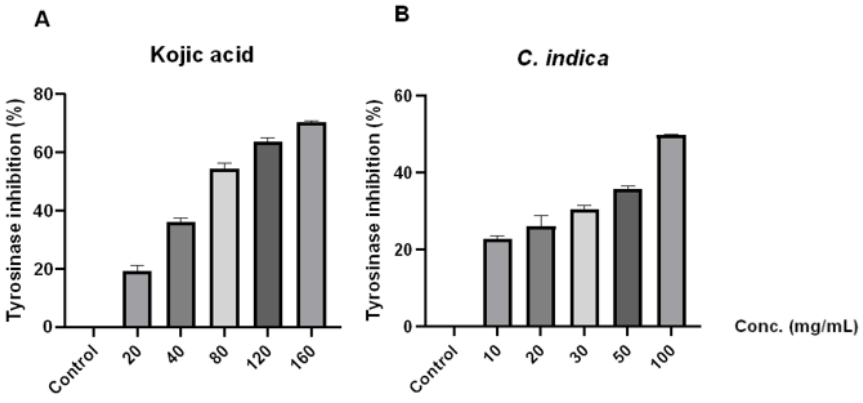


Figure 2. Tyrosinase inhibitory activity of kojic acid (positive control) (A) and *C. indica* inflorescence and leaf extract (B). The data was showed as mean ± SD of three independent experiments performed in two technical replicates. All results were significant at $p < 0.001$ compared to the control using the One-Way ANOVA.

Conclusion

The present data demonstrated that the water-soluble fraction of the ethanolic extract of *C. indica* inflorescence and leaf contained phenolic compounds and flavonoids. Moreover, the extract exerted the ability of free radical scavenging and anti-tyrosinase activity, albeit modest. These observed properties of *C. indica* are of interest as it could potentially be used as an alternative treatment for oxidative-related diseases and skin pigmentary disorders. However, the antioxidative effect and anti-tyrosinase activity of the ethanolic *C. indica* inflorescence and leaf extracts needed to be investigated in different soluble fractions. Additionally, further experiments are needed to determine the cytotoxicity and detailed mechanism of action at a cellular level.

Acknowledgement

The authors would like to thank Mrs. Piyanee Rattanachamnong, Miss Pariyachat Wiangwiset, Miss Kannapat Narasuwan, and Dr. Karnsasin Seanoon for their technical supports.

References

1. D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. Int J Mol Sci. 2016;17(7).
2. Matoba Y, Kihara S, Bando N, Yoshitsu H, Sakaguchi M, Kayama K, et al. Catalytic mechanism of the tyrosinase reaction toward the Tyr98 residue in the caddie protein. PLoS Biol. 2018;16(12):e3000077.
3. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;20(12):21138-56.
4. Li X, Madhukar Kudke A, Joseph Nepveux V F, Xu Y. Network-based pharmacology study reveals protein targets for medical benefits and harms of cannabinoids in humans. Applied Sciences. 2022;12(4).
5. Zou S, Kumar U. Cannabinoid Receptors and the Endocannabinoid System: Signaling and function in the central nervous system. Int J Mol Sci. 2018;19(3).
6. Bielawiec P, Harasim-Symbor E, Chabowski A. Phytocannabinoids: Useful drugs for the treatment of obesity? Special focus on cannabidiol. Front Endocrinol (Lausanne). 2020; 11:114.
7. Pollastro F, Minassi A, Fresu LG. Cannabis phenolics and their bioactivities. Curr Med Chem. 2018;25(10):1160-85.
8. Smith CJ, Vergara D, Keegan B, Jikomes N. The phytochemical diversity of commercial cannabis in the United States. PLoS One. 2022;17(5):e0267498.
9. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):279-309.
10. Şahin H, Boğa M, Sarı A. Terpenoid and fatty acid profiling along with anti-tyrosinase and anti-urease potentials of *Scorzonera pygmaea* Sibth. & Sm. an endemic plant of Turkey. Chem Biodivers. 2022;19(2):e202100758.
11. Nguyen HX, Nguyen NT, Nguyen MH, Le TH, Van Do TN, Hung TM, et al. Tyrosinase inhibitory activity of flavonoids from *Artocarpus heterophyllous*. Chem Cent J. 2016; 10:2.
12. Hwang YS, Kim YJ, Kim MO, Kang M, Oh SW, Nho YH, et al. Cannabidiol upregulates melanogenesis through CB1 dependent pathway by activating p38 MAPK and p42/44 MAPK. Chem Biol Interact. 2017;273:107-14.

13. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2017;32(1):403-25.
14. Vassallo A, Armentano MF, Miglionico R, Caddeo C, Chirollo C, Gualtieri MJ, et al. *Hura crepitans* L. extract: Phytochemical characterization, antioxidant activity, and nanoformulation. Pharmaceutics. 2020;12(6).
15. Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. Evid Based Complement Alternat Med. 2014;2014:253875.
16. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of Commiphora mollis (Oliv.) Engl. resin. BMC Chem. 2022;16(1):48.
17. Guan S, Su W, Wang N, Li P, Wang Y. A potent tyrosinase activator from Radix Polygoni multiflori and its melanogenesis stimulatory effect in B16 melanoma cells. Phytother Res. 2008;22(5):660-3.
18. Phupaboon S, Matra M, Prommachart R, Totakul P, Supapong C, Wanapat M. Extraction, characterization, and chitosan microencapsulation of bioactive compounds from *Cannabis sativa* L., *Cannabis indica* L., and *Mitragyna speiosa* K. Antioxidants (Basel). 2022;11(11).

F03

Mathematical Modeling of Proliferation and Apoptosis Pathways in Mammary Epithelial Cells during the Menstrual Cycles

Piyanut Ratphibun[1,2], Anuwat Tangthanawatsakul[3], Teerasit Termsaithong[4,6], Yaowaluck Maprang Roshom[5], Teeraphan Laomettachit[1,6,*]

[1] *Bioinformatics and Systems Biology Program, School of Bioresources and Technology,King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[2] *School of Information Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[3] *Department of Mathematics, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[4] *Learning Institute, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[5] *Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[6] *Theoretical and Computational Physics Group, Center of Excellence in Theoretical and Computational Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*

*E-mail: teeraphan.lao@kmutt.ac.th

Abstract

The development and regression of mammary epithelial tissue during the menstrual cycles driven by progesterone and estrogen over a woman's lifetime have been associated with the risk of breast cancer. The present work develops an ODE-based model to investigate the impact of periodic hormone fluctuations on the intracellular signaling of mammary epithelial cells. The main question is to understand at the molecular level how progesterone and estrogen promote cell proliferation and apoptosis. The ~28-day cycles of the hormones were modeled by periodic functions, while the complexity of molecular processes with kinetic equations. Model simulations can capture the change in molecular processes and biological phenomena that agree with experimental data. The rise in progesterone promotes the proliferation of epithelial cells, starting from day 11 and reaching its maximum on day 24 in the luteal phase. Meanwhile, estrogen induces apoptosis via caspase activation, reaching a high level on day 28. The model can be extended by considering other conditions (irregular menstrual cycles, irregular protein activities, etc.) that may lead to an imbalance between mammary epithelial cell proliferation and apoptosis.

Keywords: Caspase-3, cyclin e, estrogen, progesterone, ordinary differential equations

Introduction

Fluctuations in the ovarian hormones (e.g., estrogen and progesterone) during the menstrual cycle regulate the normal development and regression of the tissue architecture of the mammary gland. For example, the mammary epithelial cells are highly proliferative, and the epithelial tissue volume expands during the luteal phase when the progesterone level is high. Conversely, when the progesterone level becomes low and the estrogen level is increased during the follicular phase, the epithelial cells undergo apoptosis, and the tissue reverts to its basic architecture.[1]

An imbalance between the proliferation and regression of the mammary epithelial cycle has been linked to increased breast cancer risk in women.[1] For example, excessive stimulation of mammary epithelial cell growth enhances the possibility of random genetic mutations, which play a significant role in the development of cancer. To understand the factors affecting the risk, the present study aims to construct an ordinary differential equation (ODE)-based model that governs the complex interactions between molecular machineries that respond to the fluctuations of the ovarian hormones. The model equations describe the dynamic activities of signaling proteins regulating cell proliferation and cell death and the periodic functions of hormone fluctuations during the menstrual cycle.[2,3]

The complexity of molecular processes underlying the proliferation and apoptosis pathways has been captured by mathematical modeling techniques, such as ordinary differential equations (ODEs) that describe the biological phenomena from the kinetic equation theory. For example, a previous study[4] used a set of ODEs to describe the dynamic interactions between key molecular components in the G_1-S transition, including cyclin D, cyclin E, Rb, E2F, and p21. On the other hand, another study focused on the apoptosis execution explained by a caspase activation pathway.[5]

Additionally, some studies included the effects of estrogen on the proliferation of the cells. A mathematical model was previously developed to explain the mechanism by which estrogen affects proliferation in normal breast cells and early-stage breast cancer cells.[6] The authors developed an ODE-based model that described the role of estradiol in regulating the G_1/S transition. The model predicted that estradiol could induce DNA damage and delay the G_1/S transition by increasing the levels of p21 and p53. In another study, estradiol was assumed to induce the proliferation of the MCF-7 breast cancer cells; however, under the setting of the study, it was concluded that its effect would not lead to a clinically detectable size of tumor.[7]

However, none of the mentioned models investigated the proliferation and apoptosis of breast cells under the influences of both estrogen and progesterone. Therefore, the current study aims to investigate the complex interactions between signaling components in mammary epithelial cells and the ovarian hormones during the menstrual cycles. We hypothesize that the fluctuations in progesterone and estrogen levels during the menstrual cycles affect cell proliferation and death of the mammary epithelial cell population. In addition, the perturbation of the menstrual cycles and protein activities may result in the accumulation of cell mass, which may lead to tumor development.

Methods

Network reconstruction

We gathered genes and proteins in the signaling network regulating proliferation and regression of the MCF10A cell line (normal mammary epithelial cells) from the literature (**Table 1**), which is summarized as a diagram in **Figure 1**.

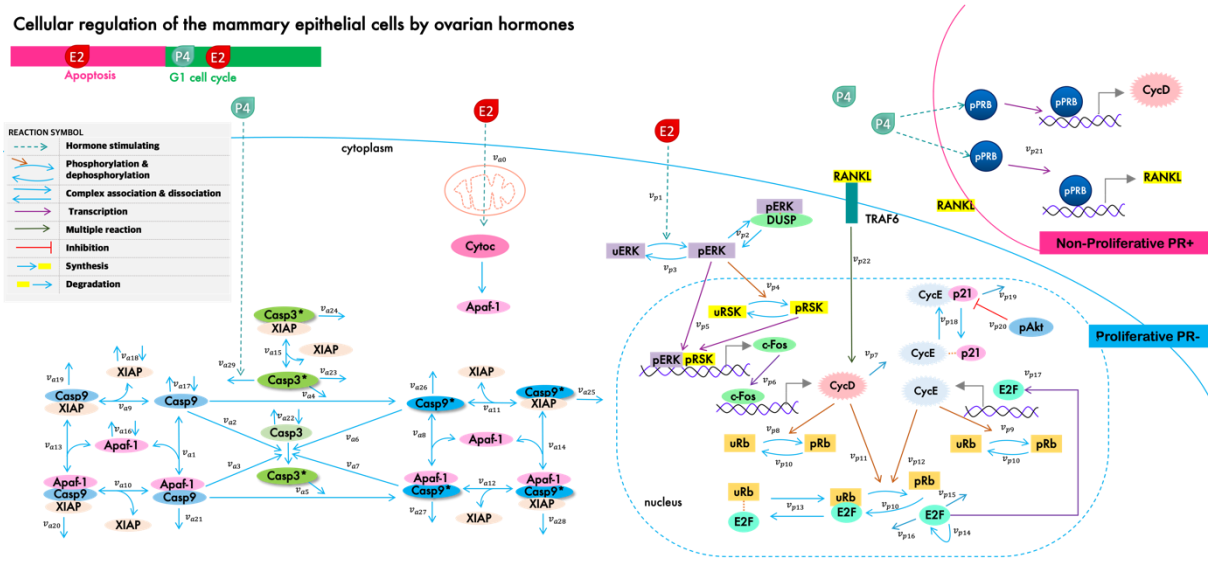


Figure 1. A schematic diagram illustrating the impact of estrogen (E2) and progesterone (P4) on the signaling network of the mammary epithelial cells. The figure includes the signaling networks of two sub-populations: progesterone receptor negative (PR−) (surrounded by the cell membrane colored in blue) and progesterone receptor positive (PR+) (surrounded by the cell membrane colored in pink).

Table 1. Abbreviations of entities in Figure 1.

Abbrev.	Full name
E2	Estrogen (17-β-estradiol)
P4	Progesterone
uERK	Unphosphorylated extracellular signal-regulated kinase
pERK	Phosphorylated extracellular signal-regulated kinase
DUSP	Dual-specificity phosphatase
uRSK	Unphosphorylated ribosomal S6 kinases
pRSK	Phosphorylated ribosomal S6 kinases
c-Fos	Protein c-Fos (proto-oncogene)
CycD	Cyclins D (D1, D2, and D3)
uRb	Unphosphorylated retinoblastoma protein
pRb	Phosphorylated retinoblastoma protein
E2F	E2 promoter binding factor (*E1A* gene)
CycE	Cyclins E
p21	Potent cyclin-dependent kinase inhibitor
pAkt	Phosphorylated Ak strain transforming
pPRB	Phosphorylation of PR-B (progesterone receptors-B)
RANKL	Receptor activator of nuclear factor-κB ligand
Cytoc	Cytochrome C
Apaf-1	Apoptotic protease activating factor 1
XIAP	X-linked IAP
Casp3	Pro-caspase-3
Casp3*	Cleaved caspase-3
Casp9	Pro-caspase-9
Casp9*	Cleaved caspase-9

Periodic functions of hormone fluctuations

The menstrual cycle patterns in women have a median cycle length of 28 days, and the highest percentage of ovulation in cycles is estimated at day 14.[8] The hormone estrogen (E2) and progesterone (P4) fluctuations were simulated by periodic functions.[3] The equations are:

$$P4(t) = pr_0 + pr_1 e^{-\frac{(t-19)^2}{pr_2}} \quad (1)$$

$$E2(t) = e_0 + e_1 e^{-\frac{(t-11)^2}{e_2}} + e_3 e^{-\frac{(t-20)^2}{e_4}} \quad (2)$$

where $P4(t)$ and $E2(t)$ are the concentration of progesterone and estrogen, respectively. pr_0, pr_1, pr_2, e_0, e_1, e_2, e_3, and e_4 are estimates for the parameters in the ovarian input functions provided by clinical data of blood levels of hormones of healthy women (**Table 2**).[3] The functions assume that the menstrual cycle period is 28 days, the ovulation date is on day 14, the highest peak of P4 is on day 19, and the first and second peaks of E2 are on days 11 and 20, respectively.

Table 2. Estimates for the parameters in the menstrual cycle input functions. Parameter values were modified from Harris LA (2002).[3]

Parameter	Value	Unit
$\boldsymbol{e_0}$	62.48	ng/L
$\boldsymbol{e_1}$	230	ng/L
$\boldsymbol{e_2}$	5	day^2
$\boldsymbol{e_3}$	115	ng/L
$\boldsymbol{e_4}$	20	day^2
$\boldsymbol{pr_0}$	0.8	nmol/L
$\boldsymbol{pr_1}$	52.24	nmol/L
$\boldsymbol{pr_2}$	19.25	day^2

ODEs of signaling proteins

The rate of each protein's activity/level change is described by an ODE with the kinetics rate laws depending on the types of biochemical reactions the protein participates in.

The proliferation pathway

We based the proliferation pathway on the model proposed by Imoto et al.[4] The model described a signaling pathway leading to the stimulation of the G_1-S transition driven by E2F and cyclin E/CDK2. We expanded the model to represent the proliferation of the mammary epithelial cells in response to estrogen and progesterone. Our model assumes that estrogen is the upstream signal of the pathway.[9] In addition, as most of the mammary epithelial cells lack the progesterone receptors, PR(−) cells, the population does not respond to P4.[10] However, it is believed that P4 induces the expression of RANKL in the PR(+) population. Then RANKL acts as a paracrine to stimulate the growth of the PR(−) population[11,12] through the phosphorylation and activation of IκB kinase (IKK)[13] , leading to IκB degradation and allowing the NF-κB (p50/p65) to enter the nucleus.[14] Inside the nucleus, NF-κB acts as a transcription factor that promotes the expression of cyclin D1.[15,16] Because RANKL activates the downstream IKK/IkB/NFkB/cyclin D1 signaling pathway[11], we simplified the multistep process into a single kinetic reaction. Therefore, in our model, P4 stimulates RANKL, which relays the signal to activate cyclin D. The model equations are shown in

Eq. (3)–(13) with reaction rates listed in **Table 3**. We estimated the model parameters from experiments measuring RANKL expression induced by P4[17] and cyclin D expression induced by RANKL.[18] Other parameter values were obtained from Imoto and Okada.[4] The full table of parameters in the proliferation pathway can be found at https://github.com/Piyanut-Ratp/MathModelMammaryCellMenstrualCycle/blob/main/01_Parameters/01_ParametersOfProliferation.csv.

Table 3. Equations for the proliferation pathway.

Index	Reaction rate
v_{p1}	$k_{onpERK} \cdot [E2] \cdot ([tERK] - [pERK])$
v_{p2}	$k_{assopERKDUSP} \cdot [pERK] \cdot [DUSP] - k_{disassopERKDUSP} \cdot [pERKDUSP]$
v_{p3}	$k_{offpERK} \cdot [pERK]$
v_{p4}	$\frac{k_{phopRSKpERK} \cdot [pERK]}{K_{MphopRSKpERK} + [pERK]} - k_{offpRSKpERK} \cdot [pRSK]$
v_{p5}	$\frac{k_{syncfos} \cdot [pERK] \cdot [pRSK]}{K_{DFos} + [pRSK]} - k_{degcfos} \cdot [cFos]$
v_{p6}	$k_{syncycDFos} \cdot [cFos]$
v_{p7}	$k_{degcycD} \cdot [cycD]$
v_{p8}	$-k_{onpRbcycD} \cdot [cycD] \cdot [uRb]$
v_{p9}	$-k_{onpRbcycE} \cdot ([tcycE] - [p21cycE]) \cdot [uRb]$
v_{p10}	$k_{offpRb} \cdot ([tRb] - [uRb])$
v_{p11}	$k_{phopRbcycD} \cdot [cycD] \cdot ([tE2F] - [E2F])$
v_{p12}	$k_{phopRbcycE} \cdot ([tcycE] - [p21cycE]) \cdot ([tE2F] - [E2F])$
v_{p13}	$-k_{assoE2FRb} \cdot ([uRb] - [tE2F] + [E2F]) \cdot [E2F] + k_{disassoE2FRB} \cdot ([tE2F] - [E2F])$
v_{p14}	$k_{synE2F} + (\frac{k_{synE2FE2F} \cdot [E2F]}{j_{synE2F} + [E2F]})$
v_{p15}	$-k_{degE2F} \cdot [tE2F]$
v_{p16}	$-k_{degE2F} \cdot [E2F]$
v_{p17}	$k_{syncycEE2F} \cdot [E2F] - k_{degcycE} \cdot [tcycE]$
v_{p18}	$k_{assop21cycE} \cdot ([tp21] - [p21cycE]) \cdot ([tcycE] - [p21cycE]) - k_{disassop21cycE} \cdot [p21cycE]$
v_{p19}	$-k_{deCycE} \cdot [p21cycE]$
v_{p20}	$-(k_{dep21} + k_{dep21cy} \cdot skp2 \cdot ([tcycE] - [p21cycE]) + pAkt) \cdot [p21cycE]$
v_{p21}	$\frac{k_{onrankl} \cdot [P4]}{K_{Mrankl} + [P4]}$
v_{p22}	$\frac{k_{oncycDrank} \cdot [rankl]}{K_{McycDrank} + [rankl]} - k_{offrankl} \cdot [rankl]$

$$\frac{d[\,pERK]}{dt} = v_{p1} - v_{p2} - v_{p3} \quad (3)$$

$$\frac{d[\,pERKDUSP]}{dt} = v_{p2} \quad (4)$$

$$\frac{d[\,pRSK]}{dt} = v_{p4} \quad (5)$$

$$\frac{d[\,Fos\,]}{dt} = v_{p5} \quad (6)$$

$$\frac{d[\,cycD\,]}{dt} = v_{p6} - v_{p7} + v_{p22} \quad (7)$$

$$\frac{d[\,uRb\,]}{dt} = -v_{p8} - v_{p9} + v_{p10} \quad (8)$$

$$\frac{d[\,tE2F\,]}{dt} = v_{p14} - v_{p15} \quad (9)$$

$$\frac{d[\,E2F\,]}{dt} = v_{p14} - v_{p16} + v_{p12} + v_{p13} + v_{p11} \quad (10)$$

$$\frac{d[\,tcycE\,]}{dt} = v_{p17} \quad (11)$$

$$\frac{d[\,p21cycE\,]}{dt} = v_{p18} - v_{p19} + v_{p20} \quad (12)$$

$$\frac{d[\,rankl]}{dt} = v_{p21} \quad (13)$$

The apoptosis pathway

The apoptosis pathway was described based on the activation of caspase induced by the mitochondria from a model proposed by Legewie et al.[5] Here, we assume that estrogen induces the cytochrome C release from the mitochondria, which acts as the upstream signal of the apoptosis pathway. On the other hand, progesterone inhibits apoptosis by deactivating caspase.[19] The model equations are shown in **Eq. (14)–(27)**, with reaction rates in **Table 4**. Most parameter values in the model were obtained from a previous model[5] when available. Other extra parameters were estimated from experiments in the literature.[20] The full table of parameters in the apoptosis pathway can be found at https://github.com/Piyanut-Ratp/MathModelMammaryCellMenstrualCycle/blob/main/01_Parameters/02_ParametersOfApoptosis.csv.

Table 4. Equations for the apoptosis pathway

Index	Reaction rate
v_{a0}	$(\frac{k_{onCytocE2} \cdot E2}{K_{MCytocE2} + E2}) - k_{offCytocE2} \cdot Cytoc$
v_{a1}	$k_1 \cdot A^* \cdot C9 - k_{-1} \cdot A^*C9$
v_{a2}	$k_2 \cdot C3 \cdot C9$
v_{a3}	$k_3 \cdot C3 \cdot A^*C9$
v_{a4}	$k_4 \cdot C9 \cdot C3^*$
v_{a5}	$k_5 \cdot A^*C9 \cdot C3^*$
v_{a6}	$k_6 \cdot C3 \cdot C9^*$
v_{a7}	$k_7 \cdot C3 \cdot A^*C9^*$
v_{a8}	$k_8 \cdot C9^* \cdot A^* - k_{-8} \cdot A^*C9^*$
v_{a9}	$k_9 \cdot C9 \cdot X - k_{-9} \cdot C9X$
v_{a10}	$k_{10} \cdot A^*C9 \cdot X - k_{-10} \cdot A^*C9X$
v_{a11}	$k_{11} \cdot C9^* \cdot X - k_{-11} \cdot C9^*X$
v_{a12}	$k_{12} \cdot A^*C9^* \cdot X - k_{-12} \cdot A^*C9^*X$
v_{a13}	$k_{13} \cdot C9X \cdot A^* - k_{-13} \cdot A^*C9X$
v_{a14}	$k_{14} \cdot C9^*X \cdot A^* - k_{-14} \cdot A^*C9^*X$
v_{a15}	$k_{15} \cdot C3^* \cdot X - k_{-15} \cdot C3^*X$
v_{a16}	$k_{-16} - k_{16} \cdot A^*$
v_{a17}	$k_{-17} - k_{17} \cdot C9$
v_{a18}	$k_{-18} - k_{18} \cdot X$
v_{a19}	$k_{19} \cdot C9X$
v_{a20}	$k_{20} \cdot A^*C9X$
v_{a21}	$k_{21} \cdot A^*C9$
v_{a22}	$k_{-22} - k_{22} \cdot C3$
v_{a23}	$k_{23} \cdot C3^*$
v_{a24}	$k_{24} \cdot C3^*X$
v_{a25}	$k_{25} \cdot C9^*X$
v_{a26}	$k_{26} \cdot C9^*$
v_{a27}	$k_{27} \cdot A^*C9^*$
v_{a28}	$k_{28} \cdot A^*C9^*X$
v_{a29}	$\frac{k_{onC3P4} \cdot P4 \cdot C3^*}{K_{MC3P4} + C3^*}$

$$\frac{d[\,Cytoc\,]}{dt} = v_{a0} \tag{14}$$

$$[\,A^{*}\,] = Cytoc \tag{15}$$

$$\frac{d[\,C9\,]}{dt} = -v_{a1} - v_{a9} - v_{a4} + v_{a17} \tag{16}$$

$$\frac{d[\,C9X]}{dt} = v_{a9} - v_{a13} - v_{a19} \tag{17}$$

$$\frac{d[\,X]}{dt} = -v_{a9} - v_{a10} - v_{a15} - v_{a11} - v_{a12} + v_{a18} \tag{18}$$

$$\frac{d[A^{*}C9X]}{dt} = v_{a10} + v_{a13} - v_{a20} \tag{19}$$

$$\frac{d[\,A^{*}C9\,]}{dt} = v_{a1} - v_{a10} - v_{a5} - v_{a21} \tag{20}$$

$$\frac{d[\,C3\,]}{dt} = -v_{a2} - v_{a3} - v_{a6} - v_{a7} + v_{a22} \tag{21}$$

$$\frac{d[\,C3^{*}]}{dt} = v_{a2} + v_{a3} - v_{a15} + v_{a6} + v_{a7} - v_{a23} - v_{a29} \tag{22}$$

$$\frac{d[\,C3^{*}X]}{dt} = v_{a15} - v_{a24} \tag{23}$$

$$\frac{d[\,C9^{*}X\,\,]}{dt} = v_{a11} - v_{a14} - v_{a25} \tag{24}$$

$$\frac{d[\,C9^{*}]}{dt} = v_{a4} - v_{a8} - v_{a11} - v_{a26} \tag{25}$$

$$\frac{d[\,A^{*}C9^{*}]}{dt} = v_{a5} + v_{a8} - v_{a12} - v_{a27} \tag{26}$$

$$\frac{d[\,A^{*}C9^{*}X]}{dt} = v_{a12} + v_{a14} - v_{a28} \tag{27}$$

Model simulation

The model was written using Python (version 3.9.10). We solved the system of ODEs by Euler's numerical integration method to yield the time-series dynamics of each variable (protein activity or level). We considered the level of cyclin E as a proxy for the proliferation activity as the protein drives the G_1/S transition that commits cells into the cell cycle. The activity of caspase-3 was considered a proxy for apoptosis.

Results

The simulation of hormone fluctuation

Figure 2 shows the fluctuation levels of the ovarian hormones over three cycles from **Eq. (1)** and **(2)** with the parameters listed in **Table 1**. Progesterone exhibited one peak on day 19 during the luteal phase of each cycle, while estrogen showed two peaks: the first peak on day 11 during the follicular phase and the second peak on day 20 during the luteal phase. The simulation results are comparable with the experimental data of the menstrual cycle period.[21]

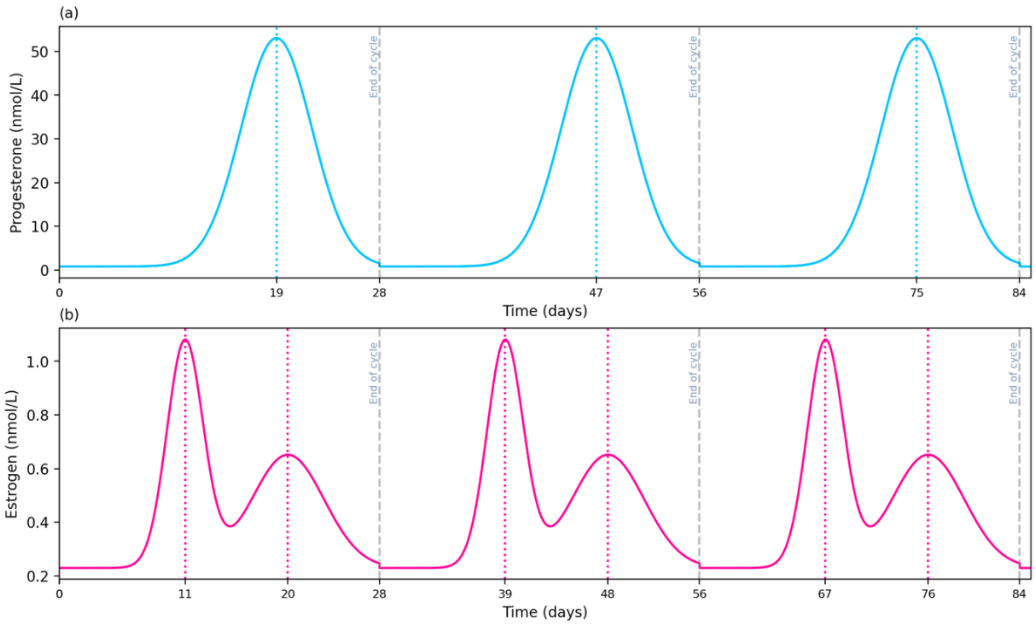


Figure 2. The fluctuations of progesterone and estrogen during 90 days of menstrual cycles using periodic functions (Eq. 1 and 2).

The simulation of the proliferation and apoptosis based on the ODEs of signaling proteins

Periodic ovarian hormones intricately regulate the downstream expression of the protein during the menstrual cycle. For example, progesterone and estrogen stimulate the mechanism of the early phase of the cell cycle (the activation of cyclin E to complete the G_1-S transition of the proliferation phase). On the contrary, estrogen stimulates the mechanism of apoptosis (the activation of caspase-3), but progesterone inhibits it. First, we studied how the proliferation and apoptosis pathways respond to the peak concentrations of estrogen and progesterone in the menstrual cycles. Therefore, we selected the hormone concentrations on days 11 and 19 as the constant upstream signals and simulated the dynamics of cyclin E and caspase-3.

In the menstrual cycle, the first estrogen peak is on day 11 (**Figure 2**) with a concentration of 1.08 nmol/L (the highest level throughout the cycle) and a concentration of progesterone at 2.65 nmol/L. The concentrations were used as the constant inputs of the ODEs to investigate the dynamic activities of cyclin E and caspase-3 in **Figure 3a** and **b**. The result showed that cyclin E accumulated and reached its steady state within 18 hours with approximately 2.74 a.u. Meanwhile, caspase-3 barely increased within 1.5 hours but abruptly activated to a high level at 3 hours and approached a steady-state level of around 131.78 nM after 7 hours.

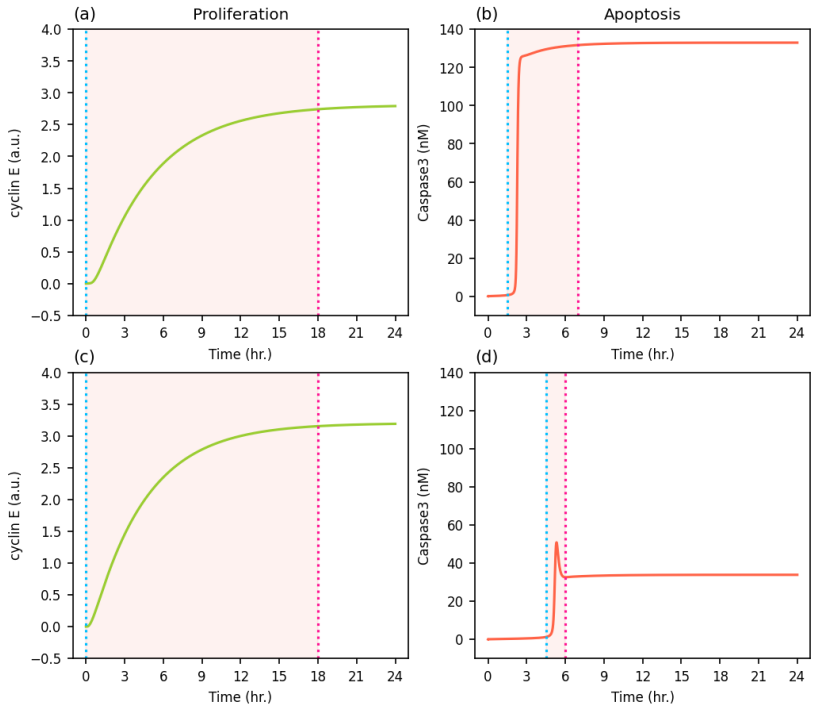


Figure 3. The dynamic activities of cyclin E and caspase-3 from the ODEs in response to the concentrations of progesterone and estrogen on days 11 and 19 of a menstrual cycle. (a, b) Cyclin E and caspase-3 respond to the hormone levels (1.08 nmol/L and 2.65 nmol/L for estrogen and progesterone, respectively) for 24 hours. (c, d) Cyclin E and caspase-3 respond to the hormone levels (0.63 nmol/L and 53.04 nmol/L for estrogen and progesterone, respectively) for 24 hours.

The second peak of the hormones is on day 19, with the highest level of progesterone at 53.04 nmol/L and the estrogen concentration at 0.63 nmol/L. The simulation in response to the concentrations (**Figure 3c** and **d**) showed that cyclin E accumulated and reached a steady state within 18 hours with approximately 3.15 a.u. Caspase-3 was activated at around 5.3 hours to approximately 50.61 nM, significantly lower than the caspase-3 level in response to the hormone profiles on day 11 (**Figure 3a** and **b**). The reduction of the caspase-3 level was due to the inhibition by progesterone.

We next simulated the model with periodic cycles of progesterone and estrogen by combining the periodic functions of hormones with the ODEs of signaling proteins to investigate the proliferative and apoptotic signals during the menstrual cycles. The result showed that the downstream signal, including RANKL, cyclin E, and caspase-3, exhibited periodic levels coordinating with the ovarian hormones (**Figure 4**). RANKL showed the highest peak on day 19 (**Figure 4a**), the same as the peak of progesterone (**Figure 2a**), because the RANKL is a direct target of progesterone (**Eq. 13**). On the other hand, the signal regulating cyclin E derives from both estrogen and progesterone (**Eq. 3–11**). Estrogen activates the downstream ERK/RSK/c-Fos/cyclin D/E2F/cyclin E signaling pathway, while progesterone activates the downstream RANKL/cyclin D/E2F/cyclin E signaling pathway (**Figure 1**). Thus, we observed cyclin E initially accumulated during day 11 due to the increase in estrogen. Then, it was continuously boosted as the increase in progesterone added more activation effects. Until day 24, when both estrogen and progesterone declined, cyclin E lowered to its basal level (**Figure 4b**). The simulated result is consistent with an experimental study reporting the mitotic activity starting around days 11 to 24[22] (orange shaded in **Figure 4**), with a peak on day 24.

In the apoptosis pathway, we assume estrogen stimulates cytochrome C, resulting in the increase in Apaf-1, which consequently increases caspase-3. The simulation result showed a slight rise of caspase-3 before day 11, following an increased estrogen level (**Figure 4c**). This result is supported by the experimental data that the breast volume dropped during days 6–12.[23] Then caspase-3 declined during the increase in progesterone from days 12 to 19 and then surged back after progesterone declined. The result can be explained by the inhibition of Fas-mediated apoptosis by progesterone, which is evident in normal breast epithelial cells.[19] A similar result with the experimental data reported that the peak value for apoptosis was at day 27.5.[22]

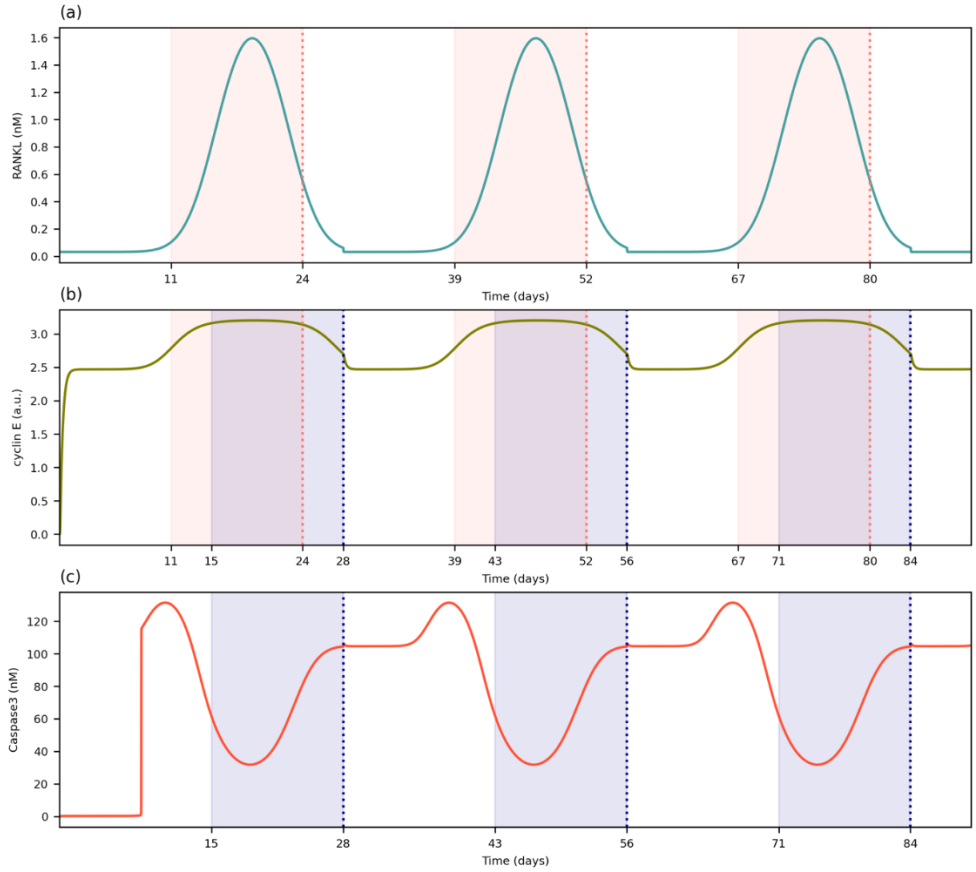


Figure 4. The fluctuation of the levels of RANKL, cyclin E, and caspase-3 regulated by progesterone and estrogen during 90 days of the menstrual cycle.

Discussion and Conclusion

We sought to develop a mathematical model explaining mammary epithelial tissue's development and regression during the menstrual cycles driven by progesterone and estrogen. Several previous studies have discussed the ovarian hormones influencing the normal breast in mammals; however, the experimental data were not apparent at the intracellular level of signal transduction. Here, we gathered biochemical interactions and parameters from the literature and used mathematical methods to depict the dynamics of molecular processes in the mammalian epithelial cells that possess biological phenomena that agree with experimental data. The rise in progesterone promotes the proliferative signal, cyclin E, by RANKL paracrine signaling and inhibits apoptosis by deactivating caspase-3.

During the proliferation phase, cyclin E serves as a representative marker of mitotic activity (indicating the G_1-S transition). Our simulation showed that cyclin E accumulated between days 11 and 24. Consistently, there is supporting data on the number of cells undergoing mitosis during days 11-24.[22] The signal of caspase-3 represented the apoptotic activity that showed a decline during days 12-19 due to progesterone accumulation. Our model assumed that progesterone decreased the apoptosis signal, which is supported by data from an experimental study.[19] Then the rise in estrogen promoted the apoptotic signal (caspase-3). The first wave of estrogen induced caspase-3 activity, but the influence of progesterone dominated until day 19. Consequently, caspase-3 was raised by the second wave of estrogen, reaching a high level on day 28. The simulation results are consistent with the reported number of cells undergoing apoptosis on day 27.5 from an experiment.[22]

In conclusion, our model demonstrated the balance between proliferative and apoptotic signals of mammary epithelial cells under the fluctuation of progesterone and estrogen during the menstrual cycles (**Figure 5**). In future work, we plan to investigate how perturbing each parameter affects the dynamics of mammary epithelial protein activities. This will help identify important factors, such as irregular menstrual cycles or irregular protein activities, affecting a balance between the proliferation and recession of the mammary epithelial cell population that may lead to tumor development. One limitation of the current model is the parameter values were mostly estimated from breast cancer cultured cell lines, which may not represent the actual values of the *in vivo* mammary epithelial cells.

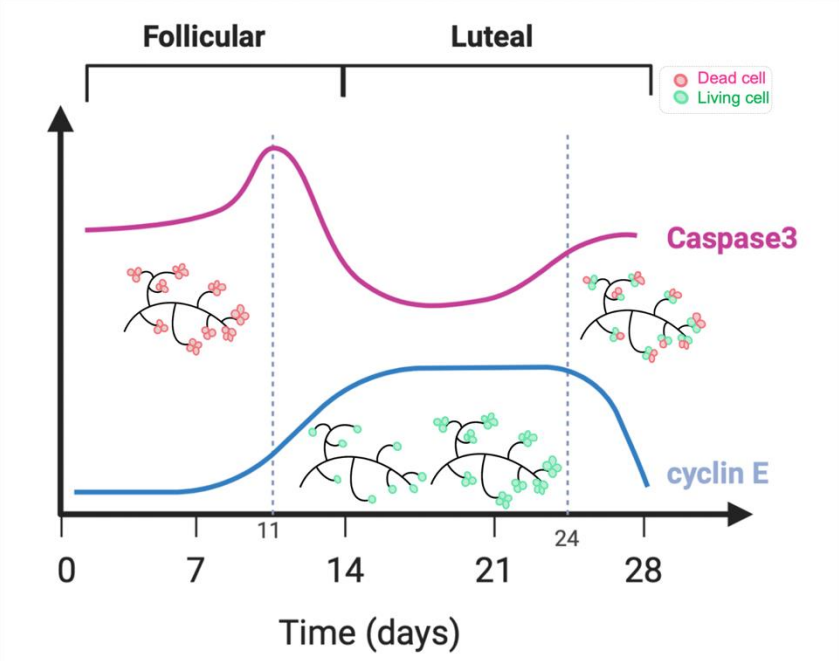


Figure 5. The summary of the fluctuations in the activities of cyclin E and caspase-3 regulated by progesterone and estrogen within 28 days relating to mammary epithelial cell proliferation (green; living cells) and apoptosis (pink; dead cells).

Acknowledgments

P.R. acknowledges the Development and Promotion of Science and Technology Talents Project (DPST). **Figure 5** was created with BioRender.com.

References

1. Atashgaran V, Wrin J, Barry SC, Dasari P, Ingman WV. Dissecting the biology of menstrual cycle-associated breast cancer risk. Front Oncol. 2016;6:267.
2. Clark LH, Schlosser PM, Selgrade JF. Multiple stable periodic solutions in a model for hormonal control of the menstrual cycle. Bull Math Biol. 2003;65(1):157-73.
3. Harris LA. Differential equation models for the hormonal regulation of the menstrual cycle [Thesis]: North Carolina State University; 2002.
4. Imoto H, Okada M. Signal-dependent regulation of early-response genes and cell cycle: a quantitative view. Curr Opin Syst. 2019;15:100-8.
5. Legewie S, Bluthgen N, Herzel H. Mathematical modeling identifies inhibitors of apoptosis as mediators of positive feedback and bistability. PLoS Comput Biol. 2006; 2(9):e120.
6. Kusuma MF, Adi-Kusumo F, editors. A mathematical modelling for estradiol influence on DNA damage response and G_1/S transition phase regulations in early stage of breast cancer. The 8th SEAMS-UGM International Conference on Mathematics and Its Applications; 2019; Indonesia.
7. Wei HC. Mathematical modeling of tumor growth: the MCF-7 breast cancer cell line. Math Biosci Eng. 2019;16(6):6512-35.
8. Grieger JA, Norman RJ. Menstrual cycle length and patterns in a global cohort of women using a mobile phone app: retrospective cohort study. J Med Internet Res. 2020;22(6): e17109.
9. Scaling AL, Prossnitz ER, Hathaway HJ. GPER mediates estrogen-induced signaling and proliferation in human breast epithelial cells and normal and malignant breast. Horm Cancer. 2014;5(3):146-60.
10. Le AV, Szaumkessel M, Tan TZ, Thiery JP, Thompson EW, Dobrovic A. DNA methylation profiling of breast cancer cell lines along the epithelial mesenchymal spectrum-implications for the choice of circulating tumour DNA methylation markers. Int J Mol Sci. 2018;19(9).
11. Obr AE, Edwards DP. The biology of progesterone receptor in the normal mammary gland and in breast cancer. Mol Cell Endocrinol. 2012;357(1-2):4-17.
12. Joshi PA, Waterhouse PD, Kannan N, Narala S, Fang H, Di Grappa MA, et al. RANK signaling amplifies WNT-responsive mammary progenitors through R-SPONDIN1. Stem Cell Rep. 2015;5(1):31-44.
13. Yamamoto M, Abe C, Wakinaga S, Sakane K, Yumiketa Y, Taguchi Y, et al. TRAF6 maintains mammary stem cells and promotes pregnancy-induced mammary epithelial cell expansion. Commun Biol. 2019;2(1):292.
14. Haga M, Okada M. Systems approaches to investigate the role of NF-kappaB signaling in aging. Biochem J. 2022;479(2):161-83.
15. Joyce D, Bouzahzah B, Fu M, Albanese C, D'Amico M, Steer J, et al. Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. J Biol Chem. 1999;274(36):25245-9.
16. Lim SY, Soh J-W. Specific isoforms of protein kinase G downregulate the transcription of cyclin D1 in NIH3T3. Bull Korean Chem Soc. 2013;34(4):1165-9.
17. Wang J, Gupta A, Hu H, Chatterton RT, Clevenger CV, Khan SA. Comment on "Progesterone/RANKL is a major regulatory axis in the human breast". Sci Transl Med. 2013;5(215):215le4.
18. Tanos T, Sflomos G, Echeverria PC, Ayyanan A, Gutierrez M, Delaloye J-F, et al. Progesterone/RANKL is a major regulatory axis in the human breast. Sci Transl Med. 2013;5(182):182ra55-ra55.

19. Behera MA, Dai Q, Garde R, Saner C, Jungheim E, Price TM. Progesterone stimulates mitochondrial activity with subsequent inhibition of apoptosis in MCF-10A benign breast epithelial cells. Am J Physiol Endocrinol Metab. 2009;297(5):E1089-96.
20. Deng Y, Miki Y, Nakanishi A. Estradiol/GPER affects the integrity of mammary duct-like structures in vitro. Sci Rep. 2020;10(1):1386.
21. Draper CF, Duisters K, Weger B, Chakrabarti A, Harms AC, Brennan L, et al. Menstrual cycle rhythmicity: metabolic patterns in healthy women. Sci Rep. 2018;8(1):14568.
22. Anderson TJ, Ferguson DJ, Raab GM. Cell turnover in the "resting" human breast: influence of parity, contraceptive pill, age and laterality. Br J Cancer. 1982;46(3):376-82.
23. Hussain Z, Roberts N, Whitehouse GH, García-Fiñana M, Percy D. Estimation of breast volume and its variation during the menstrual cycle using MRI and stereology. Brit J Radiol. 1999;72(855):236-45.

คำสั่งสมาคมเภสัชวิทยาแห่งประเทศไทย

เรื่อง แต่งตั้งคณะกรรมการจัดการประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 ปี พ.ศ. 2566

สมาคมเภสัชวิทยาแห่งประเทศไทยร่วมกับหลักสูตรสหสาขาวิชาเภสัชวิทยา จุฬาลงกรณ์มหาวิทยาลัย เป็นเจ้าภาพในการจัดประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 ระหว่างวันที่ 24-26 พฤษภาคม 2566 ณ โรงแรม แรมแบรนดท์ กรุงเทพฯ เพื่อให้การดำเนินงานการจัดประชุมดำเนินไปด้วยดี จึงขอแต่งตั้งผู้มีรายนามดังต่อไปนี้เป็นคณะกรรมการจัดการประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 ปี พ.ศ. 2566

คณะกรรมการที่ปรึกษา

1. ศ.ดร.อำนวย ถิฐาพันธ์
2. ศ. เกียรติคุณ นพ.บุญเจือ ธรณินทร์
3. รศ.ดร.ภก.ชัยชาญ แสงดี
4. พล.ต.รศ.ดร.บพิตร กลางกัลยา
5. รศ.ดร.พรเพ็ญ เปรมโยธิน
6. ดร.อุดม จันทรารักษ์ศรี
7. รศ.ดร.ภญ.มยุรี ตันติสิระ
8. รศ.ดร.ภญ.จินตนา สัตยาศัย
9. ศ.ดร.เกศรา ณ บางช้าง
10. รศ.พญ.สุมนา ชมพูทวีป
11. รศ.จันทนี อิทธิพานิชพงศ์
12. รศ.สุพีชา วิทยเลิศปัญญา
13. รศ.ดร.วัชรี ลิมปนสิทธิกุล

<u>หน้าที่</u>

ให้คำปรึกษาและสนับสนุนการดำเนินงานของคณะกรรมการดำเนินงานฝ่ายต่างๆ ให้สามารถจัดการประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 ปี พ.ศ. 2566 เป็นไปด้วยความเรียบร้อยและบรรลุวัตถุประสงค์

คณะกรรมการฝ่ายอำนวยการจัดประชุม

1.	รศ.ภญ.ดร.สุรีย์	เจียรณ์มงคล	ประธานคณะกรรมการ
2.	รศ.นพ.ดร.นิพัญจน์	อิศรเสนา ณ อยุธยา	กรรมการ
3.	รศ.สพญ.รท.หญิง ดร.เนาวรัตน์	สุธัมนาถพงษ์	กรรมการ
4.	รศ.ทญ.ดร.สิรีรัตน์	สูอำพัน	กรรมการ
5.	รศ.พญ.ดร.วรรณรัศมี	เกตุชาติ	กรรมการ
6.	รศ.ภญ.ดร.วริษา	พงศ์เรขนานนท์	กรรมการ
7.	ผศ.สพ.ญ.ดร.นิภัทรา	สวนไพรินทร์	กรรมการ
8.	ผศ.พญ.ดร.ปาจรีย์	จริยวิลาศกุล	กรรมการ
9.	ผศ.สพญ.ดร.วุฒิพร	ลิ้มประสูตร	กรรมการ
10.	ผศ.ภญ.ดร.วราลี	ยอดสุรางค์	กรรมการ
11.	รศ.ดร.ปิยนุช	วงศ์อนันต์	กรรมการและเลขานุการ
12.	นางสาววราภรณ์	มีศาสตร์	ผู้ช่วยเลขานุการ

<u>หน้าที่</u>

1. ดำเนินงานจัดประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 ปี พ.ศ. 2566 ให้เป็นไปด้วยความเรียบร้อยและบรรลุวัตถุประสงค์
2. ดูแลเรื่องเอกสารการดำเนินงานโครงการ อาทิ หนังสือขออนุมัติไม่เป็นวันลาและเบิกค่าลงทะเบียนการขอหน่วยกิตศึกษาต่อเนื่อง
3. ประสานงานการดำเนินการด้านต่างๆ ให้เป็นไปด้วยความเรียบร้อย
4. สรุปโครงการหลังเสร็จสิ้นการจัดงาน

คณะกรรมการฝ่ายวิชาการ

1.	รศ.ภญ.ดร.วริษา	พงศ์เรขนานนท์	ประธานคณะกรรมการฝ่าย
2.	ผศ.ดร.พญ.ปาจรีย์	จริยวิลาศกุล	กรรมการ
3.	ผศ.ภญ.ดร.วราลี	ยอดสุรางค์	กรรมการ
4.	อ.ภก.ดร.วิศรุต	บูรณะสัจจะ	กรรมการ
5.	อ.ภก.ดร.นนท์ธเนศ	นลินรัตน์	กรรมการและเลขานุการ
6.	นางสาววราภรณ์	มีศาสตร์	ผู้ช่วยเลขานุการ

<u>หน้าที่</u>

1. วางแผน จัดทำกำหนดการและหัวข้อการประชุม
2. ประสานงานเชิญวิทยากร
3. รวบรวมประวัติวิทยากร และเอกสารประกอบการบรรยาย
4. จัดทำระบบสำหรับการลงทะเบียนส่งผลงาน
5. จัดทำเกียรติบัตรสำหรับผู้ชนะการประกวดในการนำเสนอโปสเตอร์

คณะกรรมการฝ่ายลงทะเบียน

1.	รศ.ดร.ปิยนุช	วงศ์อนันต์	ประธานคณะกรรมการฝ่าย
2.	รศ.พญ.ดร.วรรณรัศมี	เกตุชาติ	กรรมการ
3.	อ.ดร.ศุทธินี	เผือกขาวผ่อง	กรรมการและเลขานุการ
4.	นส.วราภรณ์	มีศาสตร์	ผู้ช่วยเลขานุการ

หน้าที่

1. จัดทำระบบสำหรับการลงทะเบียน และชำระค่าลงทะเบียน
2. เปิดบัญชีออมทรัพย์เพื่อรับเงินค่าลงทะเบียนเข้าร่วมวิชาการ
3. จัดทำและดูแลการลงทะเบียนงานประชุม
4. จัดทำรายชื่อลงทะเบียนสำหรับผู้เข้าร่วมประชุม
5. จัดเตรียมใบเสร็จรับเงิน เอกสารเกี่ยวกับการเบิกจ่ายตามระเบียบให้ผู้ลงทะเบียน

คณะกรรมการฝ่ายประชาสัมพันธ์ และเทคโนโลยีสารสนเทศ (ไอที)

1.	ผศ.ภญ.ดร.วราลี	ยอดสุรางค์	ประธานคณะกรรมการฝ่าย
2.	ผศ.ภญ.ดร.สุพรรณิการ์	ถวิลหวัง	กรรมการและเลขานุการ
3.	น.ส.วราภรณ์	มีศาสตร์	ผู้ช่วยเลขานุการ

หน้าที่

1. จัดทำเอกสาร สื่อ สิ่งพิมพ์ วีดีทัศน์ เพื่อประชาสัมพันธ์โครงการประชุมฯ และดูแลการเผยแพร่ประชาสัมพันธ์ผ่านทางสื่อออนไลน์ และหน่วยงานต่างๆ
2. จัดทำระบบลงทะเบียนและส่งผลงานออนไลน์
3. ดำเนินการด้านบันทึกภาพนิ่ง ภาพเคลื่อนไหว ในกิจกรรมต่างๆของการจัดประชุม

คณะกรรมการฝ่ายต้อนรับ พิธีการ สันทนาการ และกิจกรรม

1.	ผศ.พญ.ดร.ปาจรีย์	จริยวิลาศกุล	ประธานคณะกรรมการฝ่าย
2.	ผศ.ดร.นพ.นพดล	วัชระชัยสุรพล	กรรมการ
3.	อ.นพ.คุณาธิป	นิสสัยพันธุ์	กรรมการ
4.	อ.ดร.ปุณยาภร	รัตนชีวร	กรรมการและเลขานุการ

หน้าที่

1. จัดเตรียมคำกล่าวต้อนรับ คำกล่าวเปิดและปิดงาน คำกล่าวแนะนำวิทยากร
2. จัดเตรียมพิธีกร และผู้ดำเนินรายการในกิจกรรมต่างๆ ของการประชุม
3. ดำเนินการต้อนรับและอำนวยความสะดวกแก่วิทยากร
4. ดำเนินการด้านพิธีการต่างๆ อาทิ พิธีเปิด-ปิดโครงการ การมอบรางวัล รวมถึงกิจกรรมระหว่างการประชุม
5. กำกับดูแลให้การดำเนินการด้านพิธีการและกิจกรรมการประชุมเป็นไปตามกำหนดการ

คณะกรรมการฝ่ายการเงินและจัดหารายได้

1.	รศ.ทญ.ดร.สิรีรัตน์	สูอำพัน	ประธานคณะกรรมการฝ่าย
2.	รศ.ภญ.ร.ท.หญิง ดร.ภัสราภา	โตวิวัฒน์	กรรมการ
3.	ผศ.ภญ.ดร.วราลี	ยอดสุรางค์	กรรมการ
4.	อ.ทญ.ดร.เบญจา	อิศรางกูร ณ อยุธยา	กรรมการและเลขานุการ

<u>หน้าที่</u>

1. กำหนดรูปแบบและเงื่อนไขในการสนับสนุนการประชุม
2. ประสานงานและติดตามการจัดหารายได้ และเงินสนับสนุนการประชุม การจัดทำใบเสร็จและบัญชีงบประมาณสนับสนุนที่ได้รับ
3. ประสานฝ่ายต่างๆ ในการดูแลกิจกรรมของผู้สนับสนุนการประชุมให้เป็นไปด้วยความเรียบร้อย
4. ดูแลเรื่องการยืมเงิน การเบิก-จ่าย เก็บรวบรวม ตรวจสอบและจัดทำเอกสารประกอบการเบิกจ่ายของโครงการ
5. จัดหาของที่ระลึกสำหรับวิทยากร
6. ทำรายงานสรุปค่าใช้จ่ายโครงการ

คณะกรรมการฝ่ายสถานที่ และโสตทัศนูปกรณ์

1.	ผศ.สพญ.ดร.วุฒิพร	ลิ้มประสูตร	ประธานคณะกรรมการ
2.	รศ.ภญ.ดร.รัชนี	รอดศิริ	กรรมการ
3.	น.ส.อุษณา	วงศทะยาน	กรรมการ
4.	นส.วราภรณ์	มีศาสตร์	กรรมการและเลขานุการ

<u>หน้าที่</u>

1. ดำเนินการด้านการจองโรงแรม อาหารและของว่าง สถานที่จัดประชุม ที่พักวิทยากร และส่วนลดห้องพักผู้เข้าร่วมประชุม
2. ดำเนินการเรื่องการจัดสถานที่ เวที สำหรับวิทยากร พื้นที่และบอร์ดการนำเสนอผลงานรูปแบบโปสเตอร์ ตลอดจนพื้นที่ของผู้สนับสนุนการประชุมสำหรับแสดงสินค้าผลิตภัณฑ์
3. ดำเนินการเรื่องการจัดทำป้ายชื่อและฉากหลังงานประชุม ป้ายชื่อวิทยากรบนเวทีการประชุม
4. กำกับดูแลด้านฝ่ายโสตฯ จัดเตรียมอุปกรณ์คอมพิวเตอร์ เครื่องพิมพ์ อุปกรณ์เครื่องเสียง และการเชื่อมต่อระบบออนไลน์ในการประชุม

คณะกรรมการฝ่ายเอกสารสิ่งพิมพ์ ประเมินผลและรางวัล

1. รศ.ดร.ภญ.รัตติมา	จีนาพงษา	ประธานคณะกรรมการ
2. รศ.ดร.ภญ.นันทีทิพ	ลิ้มเพียรชอบ	กรรมการ
3. รศ.ดร.ภญ.รัตยา	ลือชาพุฒิพร	กรรมการ
4. ผศ.ดร.ภญ.สกลวรรณ	ประพฤติบัติ	กรรมการ
5. ผศ.ดร.ภญ.จันทิมา	เมทนีธร	กรรมการ
6. ผศ.ภญ.อรรัตน์	โลหิตนาวี	กรรมการ
7. อ.ดร.ภญ.สุดาทิพย์	อารีชีวกุล	กรรมการ

<u>หน้าที่</u>

1. รวบรวมบทคัดย่อ และบทความของผู้เข้าร่วมงาน และจัดทำรูปเล่มแบบ E-Book และจัดทำ QR code สำหรับรูปเล่ม
2. ประสานงานกับฝ่ายวิชาการในการขอสไลด์จากวิทยากรจัดทำ QR code สำหรับเอกสารประกอบการประชุม
3. กำหนดรูปแบบการประกวดผลงานเพื่อรับรางวัล กำหนดเกณฑ์การตัดสินผลการนำเสนอผลงาน และดำเนินการด้านการตัดสินผลงาน
4. ติดต่อผู้ทรงคุณวุฒิในการประเมินบทความ บทคัดย่อ ตลอดจนการประกวดผลงานนำเสนอในการประชุมเพื่อรับรางวัล
5. ออกแบบและจัดทำใบประเมินการให้คะแนนสำหรับกรรมการตัดสิน
6. รวบรวมผลการตัดสินและประสานกับฝ่ายต่างๆในการจัดทำเกียรติบัตรและรางวัล

ทั้งนี้ให้คณะกรรมการเริ่มปฏิบัติงานตั้งแต่บัดนี้เป็นต้นไปจนสิ้นสุดการจัดประชุม

สั่ง ณ. วันที่ 26 ธันวาคม พ. ศ. 2565

(ศาสตราจารย์ ดร. เกศรา ณ บางช้าง)
นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

คำสั่งสมาคมเภสัชวิทยาแห่งประเทศไทย

เรื่อง แต่งตั้งคณะกรรมการจัดการประชุมวิชาการประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 พ.ศ. 2566 (เพิ่มเติม)

ด้วยสมาคมเภสัชวิทยาแห่งประเทศไทยร่วมกับหลักสูตรสหสาขาวิชาเภสัชวิทยา จุฬาลงกรณ มหาวิทยาลัย ได้กำหนดจัดประชุมวิชาการประจำปสมาคมเภสัชวิทยาแหงประเทศไทย ครั้งที่ 44 ในหัวข่อ “Frontier in precision medicine and targeted therapy” ระหวางวันที่ 24-26 พฤษภาคม 2566 ณ โรงแรม แรมแบรนดท กรุงเทพฯ เพื่อให้การดำเนินงานการจัดประชุมดำเนินไปด้วยดี จึงขอแต่งตั้งผู้มีรายนามดังต่อไปนี้ เป็นคณะกรรมการพิจารณาผลงานวิชาการ และการตัดสินรางวัลการนำเสนอผลงานในการประชุมวิชาการ ประจำปีสมาคมเภสัชวิทยาแห่งประเทศไทย ครั้งที่ 44 พ.ศ. 2566

คณะกรรมการผู้ทรงคุณวุฒิพิจารณาผลงานวิชาการ

1.	ศ.ดร.ภก.วีรพล คู่คงวิริยพันธุ์	คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
2.	รศ.ดร.ภญ.ลัดดาวัลย์ เส็งกันไพร	คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
3.	รศ.ดร.ภก.ศิวนนท์ จิรวัฒโนทัย	คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
4.	รศ.ดร.วรรณา ชัยเจริญกุล	วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์
5.	รศ.ดร.ภญ.มยุรี ตันติสิระ	คณะเภสัชศาสตร์ มหาวิทยาลัยบูรพา
6.	รศ.ดร.ภก.กอบธัม สถิรกุล	คณเภสัชศาสตร์ มหาวิทยาลัยมหิดล
7.	ดร.ภก.พงศธร มีสวัสดิสม	คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
8.	ผศ.ดร.ภก.สุรศักดิ์ วิชัยโย	คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
9.	ผศ.ดร.ภญ.กันตรัตน์ อรุณรุ่งวิเชียร	คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
10.	ผศ.ดร.ภก.อาณัฐชัย ม้ายอุเทศ	คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
11.	รศ.ดร.นพวรรณ ภู่มาลา มอราเลส	คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
12.	รศ.ดร.ภญ.พรพรรณ วิวิธนาภรณ์	สถาบันการแพทย์จักรีนฤบดินทร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล
13.	ผศ.ดร.วันดี อุดมอักษร	คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์
14.	ดร.สุพัตรา ลิ่มสุวรรณโชติ	คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์
15.	รศ.ดร.ณัฏฐกานต์ จิรัณธนัฐ	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
16.	รศ.ดร.นพ.ณัฐ คูณรังษีสมบูรณ์	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
17.	รศ.ดร.วุฒิไกร นิ่มละมูล	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
18.	รศ.ดร.ภก.มนุพัศ โลหิตนาวี	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
19.	รศ.ดร.ภญ.นันทีทิพ ลิ้มเพียรชอบ	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
20.	ผศ.ดร.ภก.ณัถวุฒิ แซ่ลิ้ม	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร

21. ผศ.ดร.ภก.ธนศักดิ์ เทียกทอง	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
22. ผศ.ดร.ภก.ขวัญชัย รัตนมณี	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
23. ผศ.ภญ.อรรัตน์ โลหิตนาวี	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
24. ผศ.ดร.ภญ.สกลวรรณ ประพฤติบัติ	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
25. ผศ.ดร.ภก.ดำรงศักดิ์ เป็กทอง	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
26. ผศ.ดร.ภก.ศราวุฒิ อู่พุฒินันท์	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
27. ดร.ภญ.อรนันท์ เกิดพินธ์	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
28. ผศ.ดร.ภญ.จันทิมา เมทนีธร	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
29. ผศ.ดร.ภก.เรืองวิทย์ กิจบรรณาเดช	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
30. ผศ.ดร.ภาคภูมิ ทรัพย์สุนทร	คณะวิทยาศาสตร์การแพทย์ มหาวิทยาลัยนเรศวร
31. ผศ.ดร.ภญ.วัชรี ลิมปนสิทธิกุล	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
32. ดร.ภก.นนท์ธเนศ นลินรัตน์	คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการตัดสินการนำเสนอผลงาน

ประธานคณะกรรมการ

ศ.ดร.เกศรา ณ บางช้าง	วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์

คณะกรรมการ

1. ศ.ดร.เกศรา ณ บางช้าง	วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์
2. รศ.ดร.พญ.อุไรวรรณ พาณิช	คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล
3. รศ.ดร.ภญ.วิลาสินี หิรัญพานิช ซาโตะ	คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล
4. ผศ.ดร.ศิริมาศ กาญจนวาศ	คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
5. ผศ.ดร.วันดี อุดมอักษร	คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์
6. รศ.ดร.ภญ.พรพรรณ วิวิธนาภรณ์	สถาบันการแพทย์จักรีนฤบดินทร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล
7. รศ.ภญ.จันทนี อิทธิพานิชพงษ์	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
8. รศ.ดร.วุฒิไกร นิ่มละมูล	คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
9. รศ.ดร.วรรณา ชัยเจริญกุล	วิทยาลัยแพทยศาสตร์นานาชาติจุฬาภรณ์ มหาวิทยาลัยธรรมศาสตร์
10. ผศ.ดร.ภก.ณัถวุฒิ แซ่ลิ้ม	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
11. ผศ.ดร.ภญ.สกลวรรณ ประพฤติบัติ	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร
12. ผศ.ดร.ภก.ธนศักดิ์ เทียกทอง	คณะเภสัชศาสตร์ มหาวิทยาลัยนเรศวร

ทั้งนี้ให้คณะกรรมการเริ่มปฏิบัติงานตั้งแต่บัดนี้เป็นต้นไปจนสิ้นสุดการจัดประชุม

สั่ง ณ. วันที่ 16 มกราคม 2566

(ศาสตราจารย์ ดร. เกศรา ณ บางช้าง)
นายกสมาคมเภสัชวิทยาแห่งประเทศไทย

รายชื่อผู้สนับสนุนงานประชุม

- บริษัท พริมา ไซเอ็นติฟิค จำกัด
- บริษัท RI Technologies Ltd.
- บริษัท ไบโอแอคทีฟ จำกัด
- บริษัท Rushmore Precision Co.,Ltd.
- บริษัท แบงเทรดดิ้ง 1992 จำกัด
- บริษัท ไบโอดีไซน์ จำกัด
- บริษัท จีโนแม็กซ์ เทคโนโลยีส์ จำกัด
- บริษัท ไลโฟมิกส์ จำกัด
- บริษัท เพอร์กิน เอลเมอร์ จำกัด
- ห้างหุ้นส่วนจำกัด เอ็น.วาย.อาร์
- บริษัท กิบไทย จำกัด
- บริษัทยีนพลัส จำกัด

genomax Technologies

Life Science and Clinical Research Solutions

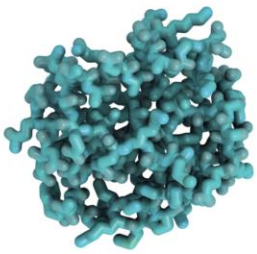
Genomax Technologies focuses on positioning solutions in five major categories of applications: bioinformatics, clinical, genomics, molecular cell biology, and proteomics, as a trusted distributor of scientific products and services that help life science research communities, biotechnology and pharmaceutical companies, clinical research organizations and hospitals plan, start, and accelerate their research.

xCELLigence RTCA eSight
Real-Time Cell Analyzer for Monitor live cell proliferation, morphology and viability

Agilent NovoCyte Flow Cytometer
Flow Cytometers systems, software, and reagents

Agilent Genomics applications and Solutions
Sample quality control (QC), NGS library preparation, microarrays, CRISPR, PCR/qPCR, bioreagents, and others

Xceler8™ Platform
Automated, rapid nucleic acid extraction



SomaScan® Assay Services
A proteomics platform for protein profiling and biomarkers discovery

X9™ Real-Time PCR System
Microfluidics-based automated platform for genotyping, gene expression, SNP, CNV, and more

We are exclusively distributor of

Agilent | Authorized Distributor

One BioMed · somalogic · STANDARD BIOTOOLS · STRECK · TheWell BIOSCIENCE · Molecular Genomics

Address: 540 Mercury Tower 8th Floor, Room 802 Ploenchit Road Lumpini, Pathumwan, Bangkok 10330 Thailand

Genomax Thailand

LINE @genomaxthailand

info@genomaxtech.com

Phone: (+66) 2 080 5663
Fax: (+66) 2 116 7676

Platinum Sponsor

Innovative Next-Generation Sequencing(NGS)

Innovative technologies to the analysis of genetic variation and function,making studies possible.

illumina® AUTHORIZED CHANNEL PARTNER


iSeq 100


MiniSeq


MiSeq


NovaSeq X Series


NextSeq 550


NextSeq 2000


NovaSeq 6000

10x GENOMICS Authorized Distributor

The Chromium X technology advantage behind single cell

analytikjena An Endress+Hauser Company

The portfolio ranges from devices for PCR, Real-time PCR, BioImaging , liquid handling, electrophoresis and sample homogenization to consumables for the whole Life Science workflow.

DeNovix®

Global leader in the development of microvolume UV-Vis and fluorescence-based instruments

DS-11 FX Series

QFX Fluorometer

MOLECULAR DEVICES

SpectraMax iD3

Multi-user microplate readers with a large touchsreen and NFC functionality

ImageXpress Pico

Compact system with intelligent image acquisition and analysis

MaxCyte®

Any Cell. Any Molecule. Any Scale. Flow Electroporation® Instruments.

Our patented Cell Engineering platform to partners engages in

- ✓ Drug Discovery & Development
- ✓ Biomanufacturing
- ✓ Cell Therapy including gene editing & immuno-oncology

expert®

AB SCIEX

Capillary Electrophoresis

Mass Spectrophotometer

BECKMAN COULTER Life Sciences

Accelerate your research with Biomek Automation

TOMY

TOMY SX-E Series

Platinum Sponsor

Bio - Active Co., Ltd.
บริษัท ไบโอแอคทีฟ จำกัด
We are your helping hands

บริษัท ไบโอแอคทีฟ จำกัด
188/1 อาคารไบโอแอคทีฟ ซอยศรีรุ่ง ถนนเชื้อเพลิง
แขวงช่องนนทรี เขตยานนาวา กรุงเทพมหานคร 10120

โทร :0-2350-3090
Email:info@bio-active.co.th
www.bio-active.co.th

@bio-active
Bio-Active Co., Ltd.
Bio-Active Channel

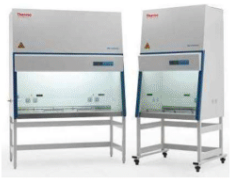
N.Y.R. limited partnership
www.nyr.co.th
Tel : 02 886 6360 ต่อ 118 Email: sales@nyr.co.th

Thermo Scientific Laboratory Equipments

Sorvall Centrifuge

Savant Speedvac

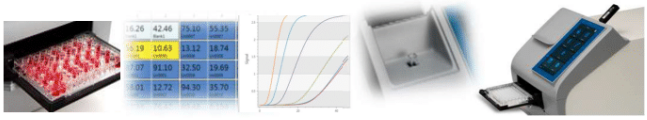
CO2 incubator



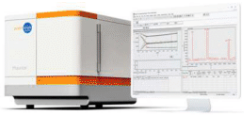
BSC class II

Thermo Scientific Microplate Readers

- เครื่องอ่านปฏิกิริยาไมโครเพลทแบบสแกนความยาวคลื่น UV-Visible พร้อมระบบควบคุมอุณหภูมิ และระบบเขย่า รองรับการอ่านปฏิกิริยาแบบต่อเนื่อง
- ควบคุมผ่านหน้าจอสีระบบสัมผัส สามารถจัดเก็บผลการอ่านไว้ในเครื่อง หรือส่งผ่านไปยัง USB memory drive ได้
- มีฟังก์ชั่นสแกนสเปคตรัม , DNA at A260 quantitation, โปรตีนทั้งแบบ colorimetric และ A280 รองรับการใช้งานกับไมโครเพลท 6-384 well และคิวเวต



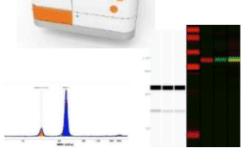
ProteinSimple Instruments



- Maurice : เครื่องวิเคราะห์เชิงปริมาณของการแยก การทำให้บริสุทธิ์และความหลากหลายในงานชีวเวชภัณฑ์ รองรับเทคนิคการวิเคราะห์ทั้งแบบ cIEF และ CE-SDS ให้ผล Native Fluorescence ควบคู่ไปกับ Absorbance A280nm ตรวจวัดได้ถึงระดับ 0.7ug/ml ให้ผลวิเคราะห์ความบริสุทธิ์ IgG ภายใน 35 นาที และแยกความแตกต่างประจุเพียงแค่ 10 นาทีเท่านั้น

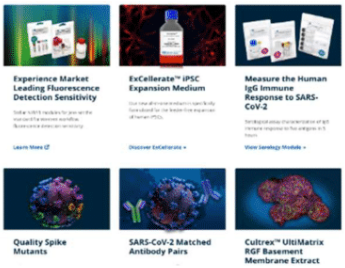
- MFI : เครื่องวิเคราะห์และถ่ายภาพอนุภาคหรือตะกอน ขนาด sub visible (1-70 ไมครอน) ในสารละลาย เช่น Vaccine, liquid drug ด้วยกล้องจุลทรรศ์ดิจิตอล ตามมาตรฐานองค์การอาหารและยาแนะนำ มีความไวสูง สามารถตรวจได้ทั้ง protien aggregate, silicone, micro-droplet, air bubble

- Jess : เครื่องวิเคราะห์แยกขนาดโปรตีน 2-440 kDa ด้วยเทคนิค Western blot แบบอัตโนมัติ ใช้เวลาเพียงแค่ 3 ชั่วโมงเท่านั้น ไม่ต้องเตรียมเจล ไม่มีขั้นตอนถ่ายโปรตีนลงเมมเบรน วิเคราะห์โปรตีนแบบ SDS-PAGE, Immunoprobe: Chemiluminescence + Fluorescence detection, protein normalization และ Re-probe



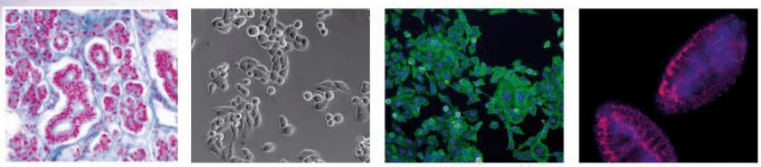
Reagents: R&D systems / NOVUS / Tocris reagents

- **Antibodies (Research/ GMP) :** Flow Cytometry, IHC/ICC/IF,WB, ELISA, **Recombinant Proteins, GMP Protein**
- **Cell culture media, supplements, growth factors, Organoid and cellular matrix for 3D Cultures**



Gold Sponsor

RUSHMORE PRECISION



ZEISS SARTORIUS i-SOLUTION Total Imaging Solution IMT i-Solution Inc. www.IMT-Solution.com ADS Biotec YOUR PATH TO DISCOVERY MetaSystems LINKAM QIMAGING

www.prima-sci.com

PS

บริษัท พริมา ไซเอ็นติฟิค จำกัด
PRIMA SCIENTIFIC CO.,LTD.

abcam Thermo SCIENTIFIC nanoString fisher scientific

AdvanceLab ACD ADVANCED CELL DIAGNOSTICS, INC azure biosystems BIO BASIC Quality-Affordable Research Bankpeptide

ExtraGene Genetics FAVORGEN BIOTECH CORP. INNOPSYS EuroBioConcept

PRIMER DESIGN KAPABIOSYSTEMS ARRAYJET sptlabtech

Gold Sponsor

Prima Scientific Co., Ltd.

147/170-171 Arun Amarin, Bangkok Noi, Bangkok 10700

Tel : 02-435-7434 , 02-434-3771 , 02-433-2081 Fax : 02-884-6441

Journal of Basic and Applied Pharmacology
(J Basic App Pharmacol)

https://li01.tci-thaijo.org/index.php/JBAP/

Proceedings: The 44th Annual Meeting of the Pharmacological and Therapeutic Society of Thailand