IL-1
Thailand Antimicrobial Resistance (AMR) Program: One Health Approach

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Background: Antimicrobial resistance (AMR) is a continually evolving public health crisis all over the world, including Thailand, and it has become one of the biggest threats to global health. The most recent attempt of the World Health Organization (WHO) to combat AMR was launching global action plan on AMR at the 68th World Health Assembly in 2015.

Methods: Thailand AMR program as one health approach has been implementing the following 10 operational actions in accordance with WHO global action plan on AMR since 2013. They are: 1) estimate national AMR burden, 2) determine AMR prevalence and AMR chain in Thailand, 3) develop national AMR containment and prevention governance, 4) develop laboratory and information technology systems for surveillance of AMR, antibiotic use and hospital-acquired infections, 5) regulate use and distribution of antibiotics in human and food animal, 6) design AMR containment and prevention campaign, 7) generate local evidence for promoting responsible use of antibiotics and infection prevention and control practices, 8) create AMR campaign package, 9) implement AMR campaign package in pilot community, and 10) conduct research and development of AMR surveillance, diagnostics, therapy and prevention of AMR infections.

Results: AMR burden in Thailand included 87,000 new AMR infections, additional 3 million days of hospital stay, and 38,000 deaths per year with the annual cost of AMR infections at 0.6% of GDP. AMR campaign packages for health professional and laypeople were developed according to the established AMR chain, AMR prevalence, and locally-generated evidence on responsible use of antibiotics and infection prevention and control (IPC). Implementation of AMR campaign packages in 4 pilot communities revealed effectiveness of AMR campaign packages in terms of improving awareness and understanding of AMR, responsible use of antibiotics and compliance to IPC practices.

Conclusion: The magnitude of Thailand AMR burden is recognized. Effectiveness of AMR campaign is demonstrated. Many AMR containment and prevention measures from Thailand AMR program are adopted as national policy and have been implemented at national scale.

Keywords: Thailand, Antimicrobial Resistance, One Health
Since the first *Legionella* strain was isolated from a patient’s autopsy tissue from an outbreak in Philadelphia in 1976, the epidemiological and clinical features of *Legionella* infection have been investigated worldwide. To date, the Chinese Wanfang Database lists 1762 Chinese articles on *Legionella*, while 236 English articles or abstracts about *Legionella* research in China can be found in PubMed. The contamination rate for *Legionella* in air-conditioning or water systems is from 10.48–54.2%. A study, by pulse field gel electrophoresis, on the homology of *Legionella* strains isolated from clinical cases and environmental water revealed genetic polymorphisms. ST1 was the most prevalent sequence type of environmental *L. pneumophila* serogroup 1 isolates found by sequence-based typing. The first report of pneumonia caused by *Legionella* in China was in 1982. National survey data showed that *Legionella* infection accounted for 5.08% of community-acquired pneumonia cases. A seroepidemiological investigation demonstrated that *L. pneumophila* is still the most common species, as found for other countries, with the predominant *L. pneumophila* serogroups being 1 and 6 in patients, with constituent ratios of 43.82% and 17.69%, respectively. Eleven outbreaks of *Legionella* infection have been reported in China, totaling 224 patients. Of these patients, 42 (18.8%) presented with pneumonia, and 182 (81.2%) presented with Pontiac fever, while two patients died. Chinese researchers found that LegK1 is a bacterial effector that directly activates the host’s NF-κB signaling pathway and likely plays important roles in modulating macrophage defense or inflammatory responses during *L. pneumophila* infection. Another study has revealed that ClpP-deletion impairs the virulence of *L. pneumophila* and the optimal translocation of effector proteins. Further basic and clinical research concerning *Legionella* infection need to be undertaken in China in future.

**Keywords:** *Legionella* research; China
IL-3

PHYLOGENETIC ANALYSIS OF EUPHORBIA PALLASII USING 5.8S RIBOSOMAL GENE, UNIVERSAL PRIMER SET OF THE ITS REGION NUCLEAR DNA SEQUENCE

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The monophyly and phylogenetic relationships of the tribe Euphorbia pallasii Turczaninow (Euphorbiaceae) were evaluated using separate weighted parsimony analyses of nucleotide sequences of the nrDNA internal transcriber spacer (ITS) region. The study included 44 in group species representing nearly all of the previously recognized genera, subgenera and sections within Euphorbeae and outgroup Lycopodium. Taxa, voucher information, and GenBank numbers for all 295 ribosomal 5.8S sequences used are listed in the Appendix. DNA extraction, polymerase chain reaction (PCR) ITS region amplification and sequencing. To compare presented Euphorbia Pallasii HSUM, E. hylonoma-99%, E. komaroviana-97%, E. pallasii EU659769-87% identical from NCBI/nblast result. We consider growing in Mongolian end other Euphorbia pallasii depend else from subgenus Euphorbiaceae.

Results: The ITS regions of the Euphorbia pallasii samples were sequenced. The universal primer set of the ITS region, ITSp1, and ITS4R amplified the PCR products with size of around 850 bp. The resulting sequence 5.8S ribosomal gene ITS region Euphorbia pallasii liner DNA nucleotide sequence statistics: information is length 806bp; single-stranded weight-246,846 kDa; double-stranded weight-492,893kDa, nucleotide distribution is Adenine (A)-174, Cytosine (C)-236, Guanine (G)-242, Thymine (T)-153, Pyrine (P)-1, A+T=0.406 and C+G=0.593 frequency. This nucleotide sequence have a 2 negative ORF (table) Restriction site is Single-cutters 4 (BamHI, EcoRV, SalI, SmaI), don’t have a Non-cutters, Double-cutters, Multipe-cutters restriction site. We has comported Euphoria pallasii 44 different regions from the plant nuclear genomes, this now shows clear support for the following relationships among the five main clades of Euphorbia: include of subgenus Esula 20 nuclear data, subgenus Rhizanthium, Euphorbia - 5, and subgenus Chamaesyce 10 sequences. Euphorbia pallasii synonyms three.

Analyses of 5.8S ribosomal RNA gene; The 5.8S ribosomal RNA gene ranged in length from 359 (Euphorbia paralias) to 1175 bp (Euphorbia hylonoma), but most accessions fell between 435 and 680 bp. Large deletions in the spacer (13 aligned bp) occurred among these was a large synapomorphic deletion of 360 bp (relative to aligned length; 13 bp inferred absolute length compared with sister group, Euphorbia four species) uniting some members of inaperturate Euphorbia (see Fig. 4, subclade C2). The 5.8S matrix consisted of 44 sequences, and the aligned length of 909 sites was 1.73 longer than the absolute length of the longest component sequence. Combined; The combined analysis represents the best overall picture of phylogenetic relationships in the family and the one on which our discussion is focused, except if taxa were only sampled for one
of the single-gene analyses. This time in the GenBank include five species Mongolian’s Euphorbia genera (Euphorbia lunulata, E. discolor, E.humifusa, E. pallasii, E. tschuiensis), Euphorbia humifusa was inscribed subgenera Chamaesyce, E. lunulata is subgenera Esula, E.discolor, E.pallasii and E.tschuiesis has uncertain. In the Mongolia don’t crowing subgenera Euphorbia, Rhizantium. Euphorbia pallasii supported relationship for Euphorbiaceae among the relate to subgenera delimitation. Main clade of Euphorbia Pallasii clade subgenus Esula is the sister group to clade subgenus Rhизanthium that is in turn sister to both clade.

**Conclusion:** Euphorbia pallasii HSUM nuclear genomic is nucleotide sequence contained of length 806 bp, Single-cutters restriction sites four (SalI, BamHI, EcoRV, Smal), two negative open reading frame; protein translated sequence include 268 amino acid, To compare presented Euphorbia Pallasii HSUM 97-99% identical from NCBI/nblast result all synonym name. We consider growing in Mongolian end other Euphorbia pallasii depend else from subgenus Euphorbiaceae. Mongolian Euphorbia genera observes presented 5 speices contained more relationship for subgenera Esula.

**Keywords:** Internal transcribed spacer; Nucleotide sequences Phylogenetic analysis, Euphorbiaceae Euphorbia pallasii
Background: To investigate whether Stat5 is a true player for cardioprotective effect on remote ischemic preconditioning (RIPC), and explored the role of Stat5 in electro-acupuncture (EA) pretreatment against myocardial I/R, and determine its molecular mechanisms.

Methods: Stat5^fl/fl^ mice and Stat5-cKO mice were randomly divided into 6 groups: Stat5^fl/fl^+I/R and Stat5-cKO+I/R groups (30/180 min left coronary artery occlusion/reperfusion); Stat5^fl/fl^+RIPC+I/R and Stat5-cKO+RIPC+I/R groups (applied RIPC with three 5-min cycles of femoral artery occlusion and reperfusion before I/R); Stat5^fl/fl^+EA+I/R and Stat5-cKO+EA+I/R groups (subjected to 20 min/day of EA treatment for 1 week before I/R). The heart tissue and blood were harvested at the end point of I/R, and the parameters were measured.

Results: RIPC activated STAT5 in the heart subjected to I/R. Decreased expressions of Cyt, cleaved caspase-3, and increased Bcl-xL and Bcl-2 were detected only in the presence of STAT5. RIPC increased cardiac HIF-1α, IL10, p-PI3K, and VEGF proteins only in the Stat5^fl/fl^ mice with RIPC. EA reduced myocardial infarct size and TUNEL-positive cells in both presence and absence of STAT5, suggesting its Stat5-independency. RNA-seq data further provided evidence that both RIPC and EA pretreatment activated multiple pathways related their protective role against myocardial I/R injury.

Conclusion: RIPC-induced protection against myocardial I/R injury was Stat5 dependent, and was through anti-apoptotic signaling and survival signaling. The cardioprotection induced by EA was Stat5-independent, but Stat5 does participate in the event through anti-apoptotic and survival signaling at certain extent.

Keywords: RIPC, Electro-acupuncture pretreatment, Stat5
Nowadays, Minimally invasive surgery including endoscopic surgery, laparoscopy and robotic assisted surgery has been replaced the open surgery due to its less invasiveness. The progress of robotic assisted surgery has been made in integrating robotic technologies with surgical instrumentation.

Evolution of the da Vinci system leads the progression of robotic surgery. The advantages of da Vinci system are 3D high definition view, endowrist instrument for the precise dissection and intuitive motion with tremor filtration. Robotic surgery has demonstrated improvement in technique and clinical outcomes as evidenced by the many thousands of successful robot-assisted cases especially in radical prostatectomy. It remains to be seen where these benefits will outweigh the associated costs over the long term.
Amyotrophic lateral sclerosis (ALS) is one of the most rapidly progressive neurodegenerative disorders of unknown causes. More than 50 randomized controlled trials (RCTs) of proposed disease-modifying drugs have failed to show positive results in the past half-century.

Clinical and postmortem observations, as well as genetic studies, demonstrate that there is considerable variability in the phenotypic expression of ALS. Upper and lower motor neuron involvement is variable in ALS, and yields a spectrum with primary lateral sclerosis and progressive muscular atrophy at the both two ends. Apart from these main subtypes, two other forms have been recognized since the late 19th and early 20th centuries, these being the flail arm (FA) and flail leg (FL) syndromes. Among these clinical phenotypes, median survival in FA and FL are twice as long as bulbar and limb onset. In addition, FA and FL could fulfill the ALS El Escorial criteria and enrolled in RCTs. One of the ways to get positive results is to make RCTs inclusion criteria strictly; however, ALS plateaus and small reversals are commonly seen, especially over brief intervals. Over 6 months, 25% of ALS participants did not decline in motor function. Thus, ALS is a heterogeneous disorder, the most obvious deficiency in clinical trials is the lack of objective biomarkers. The present clinical trials cannot distinguish those ALS patients who responded to did not respond to the drug due to the lack of an ALS biomarker, which may have resulted in an ambiguous evaluation. The identification of a simple biomarker and a biomarker-based clinical trial in ALS is needed to offer a solution to this impregnable dilemma.
Acinetobacter baumannii is a Gram-negative cocccobacillus that has emerged as an important hospital-acquired pathogen worldwide over the past three decades. This organism is recognized as one of the high-priority infectious problem causing substantial mortality prolonged hospital stay, long-term disability and additional financial burden for health systems. A. baumannii belongs to belonging to the Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex, the Acinetobacter genomospecies most commonly found in clinical specimens. Even A. baumannii is considered the species most clinically relevant and most frequently resistant to multiple classes of antimicrobial agents, the role of Acinetobacter nosocomialis and Acinetobacter pittii which belong to the ACB complex, is increasingly recognized. Epidemiological and clinical studies of Acinetobacter spp. therefore often investigate the A. calcoaceticus-A. baumannii complex as a single entity, which is practical but limits the ability to differentiate the clinical features of infection due to A. baumannii and non-baumannii ACB complex isolates.

Carbapenems are widely used to treat infections caused by multidrug-resistant (MDR) A. baumannii, and several studies have reported significant associations between the carbapenem susceptibility of the infecting isolates and clinical outcomes. Carbapenem-resistant A. baumannii (CRAB) infection is one of the most important worldwide healthcare associated diseases. Broad spectrum antibiotics have been identified as risk factors associated with acquisition of CRAB. Antibiotics use has generally been recognized as the main driving factor causing these organisms to become resistant. These hypotheses are supported by several mathematical modeling and computer simulations as well as observational studies. Then many strategies have been proposed to curtail the inappropriate use of antibiotics and reduce the duration of antibiotic exposure.

The longitudinal epidemiology of A. baumannii at Songklanagarind Hospital showed nine different sequence types involved, all of which belonged to ICL-II. \( blaoXA-23 \) was the most common acquired carbapenemase gene among carbapenem-resistant isolates. The spatiotemporal plots showed that infections were clustered, to a greater extent, by genotype across several hospital units, and, to a lesser extent, by hospital unit, especially in ICUs and respiratory care units. The evidence of within- and between-ward outbreaks from this spatiotemporal analysis indicates the need to improve A. baumannii control in this setting.

Colistin, a polymyxin antimicrobial agent, is active in vitro against most CRAB strains and is commonly used to treat patients infected with this organism. Theoretically,
intravenous administration of colistin to treat pneumonia or meningitis should not be efficacious because the penetration of colistin through epithelial lining fluid (ILF) of lung and cerebrospinal fluid (CSF) is poor with studies showing approximate 10% of the serum concentration of colistin in the ILF and CSF. Then direct administration of colistin to infected site is increasing concerned. We reported the efficacy and safety of intrathecal and intraventricular administration of colistin for meningitis and ventriculitis as well as nebulization of colistin for ventilator-association pneumonia due to CRAB.
KL-4

Searching for a Better Way to Promote Educational Internationalization
—Exploration and Practice of Educational Internationalization of Kunming Medical University

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After 84 years development, Kunming Medical University has developed a well-established medical talents training system with integrated full-time higher education (bachelor, master, doctor degree programs), further education, continuing medical education and international student education as a whole. In the past five years, the university has steadily carried forward foreign exchange and cooperation activities and achieved remarkable achievements, which effectively promoted the reform and development of the university.

Under the national strategic background “One Belt, One Road” and based on the regional location of Yunnan Province, infrastructure, teaching conditions, and advantages of Kunming Medical University, KMU has been actively engaging in exploration and practice of educational internationalization. Through the ways of pushing forward reciprocal international education exchange to improve the quality of foreign students education; perfecting mechanism and improving the level of foreign students education; deepening international cooperation in fields of education and scientific research; enriching cultural exchanges and promoting international understanding; expanding international cooperation and achieving win-win collaboration; implementing educational cooperative plan with countries by the support of “One Belt and One Road" strategy; strengthening cooperation among Toho University, Prince of Songkla University, Chiang Mai University and Kunming Medical University to promote international talents education, KMU is on the way to the building itself to be one of the top universities in western China which has high quality of talent training and the ability to serve the overall regional economic and social development, as well as strong influential capacity in South and South East Asia. Meanwhile, those practice also provides a useful reference on promoting educational internationalization for higher education institutions.

Keywords: educational internationalization    Cooperation    Quality
S-I-1

Risk factors for in-hospital shunt thrombosis and mortality in patients weighing less than 3 kg with functionally univentricular heart undergoing a modified Blalock-Taussig shunt†

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Background: To determine the association between several perioperative variables and in-hospital shunt thrombosis and mortality in patients weighing less than 3 kg with functional univentricular heart (UVH) who underwent modified Blalock–Taussig shunt. Methods: Between January 2006 and February 2016, 85 patients who weighed less than 3 kg with functional UVH and underwent modified Blalock–Taussig shunt were reviewed. In-hospital shunt thrombosis and mortality were the primary outcomes. The associations between perioperative variables and outcomes were assessed with univariate and multivariate analyses.

Results: In-hospital shunt thrombosis was 14% (12 of 85). Hospital mortality was 18% (15 of 85), which resulted in an 82% discharge survival rate. Shunt thrombosis was significantly associated with in-hospital mortality (odds ratio 18.9, 95% confidence interval 4.5–78.9). There were no statistically significant associations between weight, specific diagnosis of functional UVH and shunt thrombosis or mortality. Multivariate analysis identified delayed initiation of anticoagulant ($P < 0.01$) and postoperative cardiac arrest ($P < 0.01$) as risk factors of shunt thrombosis, while intraoperative bradycardia ($P < 0.01$), high postoperative haemoglobin ($P = 0.03$) and shunt thrombosis ($P < 0.01$) were risk factors for hospital mortality.

Conclusions: In this high-risk group of patients who weighed less than 3 kg with functional UVH and who underwent modified Blalock–Taussig shunt, in-hospital mortality was strongly associated with the occurrence of shunt thrombosis. Our study highlighted the perioperative variables of delayed postoperative initiation of anticoagulant, cardiac arrest and the occurrence of intraoperative bradycardia that were significant risk factors for shunt thrombosis and mortality. Achieving better quality of perioperative care potentially improves outcomes.

Keywords: Modified Blalock-Taussig shunt, Univentricular heart, In-hospital shunt thrombosis, Mortality

† Presented at the 30th Annual Meeting of the European Association for Cardio-Thoracic Surgery, Barcelona, Spain, 1–5 October 2016.
**Background:** In cases of synthetic vascular replacement of the complete aortic arch using an open stent graft (OSG) for the treatment of aortic arch aneurysm, and if such a procedure would be attempted, there is concern for cerebral or spinal infarction due to embolization of plaque or thrombi inside the aneurysm. We therefore reviewed the achievement of accurate positioning by introducing the OSG into the aorta under endoscopy and hereby report the results.

**Methods:** The subjects were 11 patients who underwent the procedure between April 2015 and April 2017; age was 66.6±9.4 years, and sex ratio was 10 males and 1 female. During the procedure, a unilateral axillary artery and unilateral femoral artery were exposed, a midline incision of the sternum was made, the ascending aorta was perfused, and blood was removed from the upper and lower vena cava. At a rectal temperature of 28°C, selective antegrade cerebral perfusion of the right brachiocephalic artery (BCA), the left common carotid artery (LCA) and the left subclavian artery (LSCA) was made. LSCA was ligated, and an endoscope was introduced from the peripheral transected part while the circulation was stopped, and after observing the shape of the aorta, the plaque inside the aorta and the condition of the peripheral implantation site, the OSG was inserted in the target aorta. After stump formation, conventional vascular replacement of the aortic arch was performed.

**Results:** Post-operative CT showed no cases of type 1b endoleak, no additional procedures were necessary and no complications.

**Conclusions:** Aortic endoscopic aid under circulatory arrest is very useful for the OSG method.

**Keywords:** Total arch replacement, Open stent grafting, Aortic endoscopy
S-I-3

Tips for precise laparoscopic liver resection

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Background: Liver resection has been demonstrated as the preferable initial treatment for patients with resectable liver tumor. Since 1990's, we have suggested that the laparoscopic liver resection (LLR) can provide favorable outcome with early postoperative recovery in selected patients received Minor LLRs, such as left lateral sectionectomy or partial hepatectomy in antero-lateral region of the liver. Development of instruments and technical refinement with effective usage of magnified-caudal endoscopic view, have contributed to overcome limitation of LLR. With accumulation of our experience, advanced liver procedures, such as hepatectomy for tumor located postero-superior region of the liver or anatomy oriented resections including Major LLRs have been utilized by totally laparoscopic approach.

Surgical technique: All fundamental technique to perform LLR, such as maintenance of operative field, choice and appropriate use of instruments, isolation and division of vessels, control hemostasis could be learned in Minor LLRs. Hilar dissection of hepatic inflow vessels is performed in anatomical liver resection. In our concept, individual isolation approach is used for hemi-hepatectomy, and Glissonian pedicle approach is used for anatomical hepatectomy smaller than hemi-hepatectomy. As well as technical standardization, surgical simulations using recent modalities, such as 3D-CT, intraoperative contrast enhancement US and Indocyanine green fluorescence imaging, can help to have perioperative decision in disease diagnosis, trocar insertion and liver parenchymal transection.

Results: Two hundred fifty patients received LLR. No severe postoperative morbidity above Clavien-Dindo Grade IIIb except one patient developed cerebral infarction, was observed.

Conclusion: We believe that benefits of LLR are not only minimally invasiveness, but also precise performance of surgery. The accumulation of contrivance in each Minor fundaments leads to major progress for reliable LLR.

Keyword: Liver resection, Laparoscopic surgery, Liver tumor
CRISPR-Cas9 mediated efficient PD-1 disruption on Macaca fascicularis primary T cells for immunotherapy

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Background: Programmed cell death protein 1/programmed death ligands 1(PD-1/PD-L1) is a critical for modulating immune responses to cancer. We aim to block PD-1/PD-L1 immune checkpoint signal with CRISPR/Cas9 system in Macaca fascicularis primary T cells for immunotherapy.

Methods: Designed 2 pairs of sgRNA according to the 5 exons regions of PD-1 whole gene to guide PD-1 gene disruption, construct combined pST1374-Cas9-N-NLS-flag-linker plasmid, and confirm the plasmid by sequence analysis, then transfected it into T cells to recruit PD-1 disruption T cells, the function of gene modified T cells and its safety assessment in vivo were all detected by FACS, ELISPOT and CCK-8 assays.

Results: It showed that the gene knockout of PD-1 by electroporation of plasmids encoding sgRNA and Cas9 was technically feasible. FACS results, ELISPOT and CCK-8 assays indicated the disruption of inhibitory checkpoint gene PD-1 resulted in significant reduction of PD-1 expression but didn’t affect the viability of Macaca fascicularis primary T cells during the prolonged in vitro culture. Cellular immune response of the gene modified T cells exhibited enhanced cytotoxicity. Furthermore, PD-1 knock-outed T cells injected via arm vein into the Macaca fascicularis for safety evaluation, there were not any acute or subacute even chronic toxic effects in vivo.

Conclusion: These results suggest that we have demonstrated an approach for efficient checkpoint inhibitor disruption in T cells, providing a new strategy for both research and clinical applications and will be beneficial to cancer adoptive cell transfer treatments using tumor-specific lymphocytes in the near future.

Keywords: CRISPR-Cas9; PD-1; immunotherapy
S-II-1

Recurrence patterns and treatment outcomes in pure and mixed type uterine papillary serous carcinoma

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Background: Uterine papillary serous carcinoma (UPSC) represents clinical aggressiveness and accounts for almost 40% of all endometrial cancer-related deaths. The aim of this study was to evaluate the post-surgical outcomes and recurrence patterns in patients with pure and mixed type UPSC after primary treatment.

Materials and Methods: A retrospective review of the records of 64 women who underwent surgery and adjuvant platinum-based chemotherapy for UPSC between January, 2001 and December, 2014. Recurrence-free (RFS) and overall (OS) survival were calculated using the Kaplan–Meier method.

Results: Recurrences occurred in the following: 6 (37.5%) women with pure UPSC versus 13(27.1%) with mixed type UPSC. The common sites of recurrence were at paraaortic lymph nodes and vaginal cuff. The median time to recurrence was 16.8 months. For patients with mixed UPSC, the 5-year OS probability was 63.9% (95%CI=44.3 to 83.5%) compared to 29.0% (95%CI=22.4 to 35.6%) for those with pure UPSC, respectively; p = 0.002

Conclusion: patients with pure UPSC had a worse prognosis compared with those with mixed UPSC. Distant metastasis was common in both pure UPSC and mixed UPSC. Both local relapse and distant or combined sites of recurrences occurred most within 2 years after primary treatment. The common site of distant relapses was paraaortic lymph node, whereas, the local relapses were commonly found at vaginal cuff.

Keywords: recurrence, uterine papillary serous carcinoma, platinum based chemotherapy
S-II-2
Going with minority: 1 case of abnormal Luminal A breast cancer in precision medicine era

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Background: In recent years, “precision medicine” becomes hot medical concept. Based on the testing results of the precision in theory, Luminal A type breast cancer is not sensitive to chemotherapy, usually it is more likely to be sensitive to endocrine therapy. However, is it really the case?

Methods: In July 12, 2012, a 48-year old woman was diagnosed with breast cancer, T3N0M0, ER(++80%), PR(++65%), Ki67( 5%), Her-2(−). Neoadjuvant chemotherapy regimen TAC 4 cycles later, the outcome was SD, then regimen NP 4 cycles later, the outcome was CR, then modified radical mastectomy, radiotherapy and endocrine therapy (TAM) was given in sequence. In Sept 7, 2013, the patient came back because of 4 nodules on chest wall, breast cancer recurrence was confirmed by biopsy of one of the nodules. IHC result: ER(++80%), PR(++70%), Ki67( 10%), Her-2(−). Letrozole was given, three months later, the outcome was PD, then regimen NP 4 cycles later, the nodules disappeared. Exemstane was given, three months later, the outcome was PD! Then regimen NP 4 cycles later, the nodules disappeared again. Fulvestrant 500mg was given. Up to now, it has been 46 months, the chest wall is clear.

Results: Since Fulvestrant 500mg was given, up to now, it has been 46 months, the chest wall is clear.

Conclusion: We should follow the treatment guideline, but we do not have to stick to it. For Luminal A breast cancer, it may not be sensitive to TAC chemotherapy, it may be sensitive to NP chemotherapy; it may not be sensitive to TAM or AIs, it may be sensitive to Fulvestrant. More patience of doctor, more chance for patient.

Keywords: Breast cancer; minority; effect
S-II-3

Novel potential biomarkers to improve treatment outcome in non-small cell lung cancer patients

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Precision medicinal approach in cancer treatment utilizes molecularly-specified genetic information to tailor treatment to the individual cancer patient for the best treatment outcome. As a transcription and replication factor, c-Myc activates as well as represses a large number of target genes that promotes and inhibits cell growth, respectively. Overexpression of c-Myc has been observed in non-small cell lung cancer (NSCLC), compared to the premalignant lesions within the same patients, highlighting the significance of this molecule during stepwise lung carcinogenesis. The copy number gain of cytoband 17q25.3 has been reported in NSCLC patients emphasizing its clinical significance as a potential molecular target for therapy in this cancer. A majority (61%) of NSCLC patients harboured clonal gains of cytoband 17q25.3 and had a tendency of poorer overall survival. The gain of 17q25.3 in conjunction with the increase of the control region 17p11.2, reflecting a gain of chromosome 17, was also observed in approximately one third (38.9%) of the patients. Molecularly targeted therapy against the epidermal growth factor receptor (EGFR) mutation in advanced NSCLC patients has been established as an effective treatment. Other novel genetic aberrations potentially targetable in NSCLC include ALK and ROS1 translocations, MET and PD-L1 expression. We, therefore, have further explored to demonstrate and validate novel molecular biomarkers and therapeutic targeted molecules to optimize and individualize therapy for NSCLC patients. c-Myc, EGFR-relating molecules (Ras, PI3K, VEGF), ALK, ROS1, MET, PD-L1 by immunohistochemistry and the cytoband 17q25.3 copy number by fluorescence in situ hybridization in 3 cohorts of NSCLC patients have been examined. The patient cohorts represented resected early-staged and advanced-staged patients receiving anti-EGFR tyrosine kinase inhibitors and/or chemotherapy. The additional results will be updated and potentially providing a better understanding of NSCLC tumorigenesis as well as therapeutic targets leading to improvements in the treatment outcome.

Keywords: biomarkers, non-small cell lung cancer, c-Myc, cytoband 17q25.3, EGFR, Ras, PI3K, VEGF, ALK, ROS1, MET, PD-L1
Pyogenic Cerebral Ventriculitis is not catastrophic anymore, the advanced management by Neuroendoscopic Surgery

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Background: Pyogenic cerebral ventriculitis is a debilitating form of intracranial infection with an unfavorable outcome due to lack of experience in surgical management.

Objective: To study retrospectively a group of pyogenic cerebral ventriculitis patients managed by neuroendoscopic surgery (NES).

Methods: The standard intraventricular protocols of NES to treat this disease included 1 or more of the following: (1) to obliterate debris, (2) to observe evidence of microbial infection, (3) to perform septomy, (4) to incise the septation, or (5) to monitor catheter insertion. Modified EVD (mEVD) was combined with NES when intraventricular debris and bacterial plaques could not be evacuated completely. Subsequent surgical treatment strategies depended on the clinical manifestation, CSF analysis, and mEVD blockage tests approximately 3 weeks after the last NES.

Results: Forty-one patients, who were distributed in 7 hospitals and underwent NES, were included. Five patients received 1 NES, 18 received 2, 16 received 3, and 2 received 4. mEVD was performed in all patients, and mean mEVD duration in the hospital was 27.6 days. At discharge, 15 patients were cured, 15 were cured but V-P shunt-dependent, 9 were mEVD-dependent, and 2 died (mean mRS was 2.48). Two mEVD-dependent patients died, and no other outcomes changed during post-operative follow-up (mean mRS was 2.67).

Conclusion: The results suggest a relatively favorable outcome for management of pyogenic cerebral ventriculitis by NES. The techniques and strategies are practical and should be applied more extensively.

Keywords: Neuroendoscopy; Prognosis; Surgery; Ventriculitis
Protection against experimental diabetic peripheral neuropathy by anti-oxidant, anti-inflammatory, and wound healing activities of okra seed extract

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Background: Peripheral neuropathy is inevitable nerve damage caused by chronic high blood sugar or diabetes. About 60-70% of patients with diabetes will eventually develop peripheral neuropathy. Its consequences can be life-threatening. An infection that won’t heal due to poor blood flow causes risk for developing ulcers and can lead to amputation or even death. Good control of diabetes over time is the key in treating diabetic neuropathy. Vegetables, which are rich in flavonoids, have been shown to reduce insulin resistance, improve insulin sensitivity, and blunt cravings.

Methods: Seeds of Abelmoschus esculentus L (okra) were dried at 60 OC for 72 h and ground to yield fine particles. One gram of the seed powder was extracted by 2 mL of absolute ethanol. The obtained extract was determined for phytochemicals, total phenolic content (TPC), in vitro biological activities including antioxidation, anti-inflammatory and wound healing, as well as cytotoxicity.

Results: Concerning GC-MS patterns, the extract was shown to contain long chain fatty acids and fatty acids esters by about 59%, as well as sterols and derivatives by approximately 40%. Different classes of compounds, such as glutathione, tannins, saponins, flavonoids, terpenoids and polyphenols were present, according to specific chemical tests. The TPC was equivalent to 4.6 mg gallic acid/g dried weight. The extract revealed DPPH radical scavenging activity of 350 µmol trolox equivalent, ferric reducing antioxidant power (FRAP) of 170 µmol Fe²⁺ equivalent, and total glutathione of 2.5 mg/g dried weight. The survival, proliferation, and migration of L929 fibroblasts pre-treated with DPPH (37.5 µmol/L, 30 min) or H₂O₂ (150 µmol/L, 4 h) were improved following these cells were incubated with 1.25 mg/mL extract for 2 days. In addition, the levels of nitric oxide (NO), tumor necrosis factor α (TNF-α), and matrix metalloproteinase 9 (MMP-9) in the cultured supernatants were decreased by such treatment.

Conclusion: Okra seed extract might be superior in prevention and treatment of oxidative stress-induced degenerative disorders, including diabetic peripheral neuropathy.

Keywords: Abelmoschus esculentus (L.) Moench, GC-MS, TPC, DPPH, FRAP, glutathione, MTT, wound healing, TNF-α, NO, MMP-9
Reversible axonal damage is remarkable in acutely worsening symptoms of compression myelopathy; analysis of cerebrospinal fluid samples

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Background: We determined levels of biomarkers reflecting damage to axon, myelin, astrocyte, and neuron in the cerebrospinal fluid (CSF) of patients with worsening symptoms of compression myelopathy.

Methods: From 2011 to 2015, we collected 69 samples of CSF from patients before spinal surgery for acutely worsening compression myelopathy (AM, 20), chronic compression myelopathy (CM, 20), and lumbar canal stenosis (LCS; control group 29). We measured levels of pNF-H and tau (reflecting axonal damage), MBP (reflecting demyelination), S100b (reflecting astrocyte damage), and NSE (reflecting neuronal damage). Change of neurological function was determined using a JOA score for cervical myelopathy.

Results: Significantly higher levels of pNF-H (pg/ml) were detected in the CSF of AM (1907.8 ± 730.4) compared with those in either CM (198.6 ± 124.5) or LCS (462.9 ± 635.1) group (P < 0.01). Significantly higher levels of tau were detected in the CSF of AM compared with CM (P < 0.05). On the other hands, the level of MBP, S100b were not significantly different between the three groups. By contrast, the levels of NSE in AM and CM were significantly lower than those in LCS (P < 0.01). In the neurological outcome, a positive correlation between pNF-H and recovery of JOA score was observed (y = 0.0072 x + 51.85; r = 0.381).

Conclusion: The present results suggest that axonal damage is remarkable compared with demyelination, astrocytic, and neuronal damage in AM. Better clinical outcome in AM patients with high CSF levels of pNF-H indicates axonal damage in the spinal cord is reversible, and the levels of pNF-H can be predictive of good surgical outcome for patients with AM.

Key word: compression myelopathy, biomarker, cerebrospinal fluid
Creative Design for Elderly with Brain Disorder

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Background: As people age, the brain and nervous system increase risk of disease. Alzheimer’s disease, Parkinson’s disease, and stroke are top rank of the disease causing high disability and impact to quality of life. Although pharmacotherapy is a key role for patient management, but managing other part of care process is an essential role.

Method: We developed the non-pharmacologic therapy projects that can be used as a model for developing care in neurological disease in elders.

Results: Two projects to improve care in neurological disease have been developed.
1. Cognitive Game: Brain exercise using game is a tool for improving cognitive function. Developing cognitive game in Thai cultural way is a collaborative project by NNC, GMC, TCDC (Thailand Creative and Design Centre), ENSCI Les Ateliers, Paris, and Faculty of Fine Art, CMU. Three types of Cognitive game were invented and tested among elders.
2. Medical home project for aging: In this project, we build up multidisciplinary team including physicians, architects, physical/occupational therapists, technologists, and creative designers, to evaluate the patients (including the patient suffered from Alzheimer’s disease, Parkinson’s disease, and physical disability), their activities, and home. The 15 families were selected to visit, and generate the model of activities design, and home and environment improvement.

Conclusion: Our projects of care for neurological disease could be used as a prototype to develop the better care other than pharmacologic therapy.

Keywords: Neurological disorder, Aging, Cognitive game, Home therapy
Minocycline-suppression of early peripheral inflammation reduces hypoxia-induced neonatal brain injury

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While extensive studies report that neonatal hypoxia-ischemia (HI) induces long-term cognitive impairment via inflammatory responses in the brain, little is known about the role of early peripheral inflammation response in HI injury. Here we used a neonatal hypoxia rodent model. Then, an initial dose of minocycline (45 mg/kg) was injected intraperitoneally (i.p.) 2h after the hypoxia exposure ended, followed by half dosage (22.5 mg/kg) minocycline treatment for next six consecutive days daily. Saline was injected as vehicle control. To examine how early peripheral inflammation responded to hypoxia and whether this peripheral inflammation response was associated to cognitive deficits. We found that neonatal hypoxia significantly increased leukocytes not only in blood, but also increased the monocytes in central nervous system (CNS), indicated by presence of C-C chemokine receptor type 2 (CCR2⁺)/CD11b⁺CD45⁺ positive cells and CCR2 protein expression level. The early onset of peripheral inflammation response was followed by a late onset of brain inflammation that was demonstrated by level of cytokine IL-1β and ionized calcium binding adapter molecule 1(Iba-1; activated microglial cell marker). Interrupted blood-brain barrier (BBB), hypomyelination and learning and memory deficits were seen after hypoxia. Interestingly, the cognitive function was highly correlated with hypoxia-induced leukocyte response. Notably, administration of minocycline even after the onset of hypoxia significantly suppressed leukocyte-mediated inflammation as well as brain inflammation, demonstrating neuroprotection in systemic hypoxia-induced brain damage. Our data provided new insights that systemic hypoxia induces cognitive dysfunction, which involves the leukocyte-mediated peripheral inflammation response.

Keywords: Neonatal hypoxia; Hypomyelination; Minocycline
S-IV-1

The challenge in renal transplantation for small children less than 15kg

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Background: Renal transplantation in small children has the risk for early graft loss due to hypoperfusion and vascular thrombosis compared with adults. To achieve successful outcome of renal transplantation in small children, careful management must be needed.

Methods: We examined 46 pediatric patients (24 boys and 22 girls) who received a live donor renal transplant at our center between March 2009 and May 2017. The outcomes such as s-Cr (mg/dl) after transplantation, graft survival rate and patient survival rate were evaluated.

Results: In recipients, age was 4.5±2.0 years old, and weight/height were 11.7±2.2kg and 89.5±9.3cm. Donors were only relatives and the age was 38.1±7.9 years old. All but one had immediate graft function and the time of initial urine after reperfusion was 22 (interquartile range12-49) minutes. S-Cr were 0.42±0.35 at post-operative day (POD) 1, 0.27±0.08 at POD 7 and 0.29±0.09 at POD 28. The patient survival rate and graft survival rate at 1 and 5 years post-transplant were 100%/97.8% and 97.0%/ 94.8%, respectively. One recipient lost the graft due to vascular thrombosis and transplant nephrectomy was performed at 1 day post-transplant. Patient death was shown in one cases because of bacterial pneumonia at 6 months after transplantation. The others still had well-functioning graft at end of the observation.

Conclusion: The short- and long-term outcome of pediatric transplantation in small children was excellent.

Keywords: renal transplantation, pediatric transplantation
Stem cell (SC) is a cell that can proliferate and differentiate into particular cells. SC has been used in many paradigms of clinical practice. Stem cell transplantation (SCT) has been used as standard treatment for many hematologic diseases. Autologous SCT is the transplantation which use own SC and is used in multiple myeloma, relapsed and refractory lymphoma. The cell dose and viability of SC are the main key factors of successful treatment. Our faculty has the collaborative research with other faculties in the field of SC proliferation, viability and clinical application. On the other hand, allogeneic SCT use the SC from donor source. This kind of treatment successes by the combination of chemo-radiation conditioning regimen effect and immunological effect of graft versus malignancy which mediated through immune system such as T helper cells from donor. This particular research field is developed and investigated in our faculty.
The SC plasticity of mesenchymal SC has been studied in many clinical settings such as myocardial infarction and neurogenerative diseases. Our faulty is interested in the bioengineer and regenerative tissue culture studies such as the use of autologous chondrocyte transplantation and autologous SC in acute and chronic arterial insufficiency.
Recently, cellular therapy as the adoptive autologous T cells to express the chimeric antigen receptors (CAR-T cells) is emerged. These CAR-T cells have been used as a targeted immunotherapy in acute lymphoblastic leukemia with promising result. It has been developed in variety of clinical indications such as lymphoma and multiple myeloma. But the efficacy should be balance with unique side effect of the cytokine storm. The result is very promising and challenging for the use of adaptive own cells as a sort of immunotherapy for cancer treatment.

**Keywords:** Stem cell, Stem cell transplantation
Antibiotic using in pig farming and implications for public health: the case of L County

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Background: In the last four decades major changes have occurred in pig farming in rural China as the increasing application of new livestock farming technology such as new breeds, feed, vaccination and veterinary drugs, which have profound implications for public health. Among others, the application of antibiotics in pig farming is an increasing but not well regulated phenomena. Studies revealed that samples of pig manure and soil collected from big swine farms in China contained diverse and abundant antibiotic resistance genes. However, not much research was conducted to look into the antibiotic using behaviour of pig farmers. Funded by IDRC, we undertook a research in one county in Yunnan, China aiming at understanding the antibiotic using behaviours of pig farmers.

Methods: L County in Yunnan was selected as the study site where we conducted a household survey in four townships during August 2014 and April 2015 in which 404 farmer households were interviewed by using a questionnaire.

Results: Of the 404 surveyed farmers, scale farmers and smallholder farmers accounted for 21.3% and 78.7% respectively. 83.7 % of the farmers reported that they had the experience of "self-purchasing" antibiotics and 40.3% of the farmers expressed that they “often” use antibiotics in pig farming. Scale farmers are more likely to self-purchase antibiotics. Around 20% of the farmers had the experience of using human antibiotics to treat sick pigs. Scale farmers tend to use more kinds of antibiotics than backyard farmers ($\chi^2=20.180, P<0.001$) and farmers who have more knowledge on antibiotics used more kinds of antibiotics than farmers with less knowledge($\chi^2=8.567, P<0.01$). Farmers whose livestock previously suffered from disease used more kinds of antibiotics ($\chi^2=15.069, P<0.001$) and farmers who have vaccinated their pigs use more kinds of antibiotics than those who did not ($\chi^2=14.193, P<0.001$).

Conclusion: Antibiotics are widely and frequently used in both scale and smallholder pig farming that increase the risk of microbes resistance and antibiotic residues in pork products. This calls for action to reduce the irrational use of antibiotics in pig farming and to monitor antimicrobial resistance.

Keywords: pig farming; antibiotics use; behaviours
Ninty-three species of blow fly have been found in Thailand, not only in urban areas but also in the forest, especially in high mountainous regions. *Chrysomya megacephala*, *Chrysomya rufifacies* and *Lucilia cuprina* have been the most common species found in adult fly surveys. They are synanthropic flies that adapt to living in close proximity to human habitations, thereby making them likely mechanical carriers of several pathogens to humans. Some species such as *C. megacephala*, *Chrysoma pinguis*, *Chrysomya chani*, *Chrysomya thanomthini* and *Lucilia porphyrina* inhabit forest areas. Not only the adults of these species are mechanical carriers of pathogens, but their larvae also can cause myiasis in both humans and animals. From a positive point of view, blow fly larvae found in human corpses can be used as entomological evidence, thereby supporting information used in forensic investigations, such as estimating the postmortem interval, determining the cause of death, and supporting evidence of corpse relocation. Species such as *C. megacephala* and *C. rufifacies* are involved predominantly in forensic investigations, as reported in published works in Thailand and its neighboring countries. Another aspect concerning blow flies from a medical point of view is Maggot Debridement Therapy (MDT). Although *Lucilia sericata* has been used for MDT in Thailand, it has to be imported for this purpose. Other species that inhabit Thailand, such as *C. megacephala* and *L. cuprina* are being investigated currently for the possibility of application for MDT in the country. For the purposes of applying blow flies, basic information of these medically important species is mandatory. Fly identification is problematic and needs an expert taxonomist for correct identification. The molecular biology technique is used currently for adding more information and helping in fly identification in Thailand.

**Keywords:** blow fly; forensic entomology; maggot therapy; identification, molecular technique

**References:**
S-V-3
Safety and efficacy of antituberculosis drugs as dry powder inhalation

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Inhalation therapy is a promising drug delivery approach for tuberculosis treatment. However, there is always concern about the safety of the dosage form by inhalation as it may induce inflammation. Developing a new dosage form for inhalation must include tests for its safety especially for the tumor necrosis factor (TNF)-α and interleukine (IL)-1β. The safety of four anti-tuberculosis (anti-TB) drugs administered via inhalation was assessed in healthy volunteers. Four anti-TB drugs; isoniazid, rifampicin, pyrazinamide and levofl oxacin were prepared as dry powder and evaluated for uniformity of delivered dose and in vitro drug deposition. These four anti-TB dry powder formulations for inhalation met the criteria of uniformity of delivered dose and exhibited suitable size for lung delivery. Forty healthy volunteers were recruited and each was sequentially challenged with isoniazid, rifampicin, pyrazinamide and levofl oxacin in different orders. Safety was monitored by measuring the pro-inflammatory cytokines in their sputum, lung function test, blood chemistry and adverse events. This study proves that all four anti-TB dry powders did not provoke inflammatory cytokines and are safe to healthy volunteers. Now we have reached clinical trial phase II. It is expected that if the outcomes are promising then it can be translate to real life application in TB patients.

Key words: antituberculosis; clinical trial; pulmonary drug delivery; dry powder inhalation; toxicity; cytokines
Impact of antiviral therapy on the peripheral Th17/Treg cytokines and specific transcription factor (RORγt, Foxp3) in patients with chronic hepatitis B

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**Background:** Aims: To explore the impact of longtime antiviral therapy with entecavir on the peripheral Th17/Treg cytokines and specific transcription factor (RORγt, Foxp3) in patients with chronic hepatitis B.

**Patients and methods:** One hundred and thirty-six patients with chronic hepatitis B, who received entecavir therapy for 48 weeks, were enrolled the study. 20 normal individuals were as control group. Serum HBVDNA load was measured by Real-Time-PCR, and the HBV markers were detected with ELISA during 48 weeks of the treatment. Serum levels of IL-17, TGF-β, IL-10 and IL-23 were measured using an enzyme-linked immunosorbent assay (ELISA). RT-qPCR method was applied to determine the expressions of RORγt and Foxp3 mRNA of peripheral blood.

**Results:** Of the 136 patients, all HBeAg positive and with detectable HBVDNA, the majority (83.0%) had serum levels of HBVDNA over $10^7$ copies per milliliter. Chronic hepatitis B patients had significantly increased serum levels of IL-17, TGF-β, IL-10 and IL-23 compared with normal individuals ($p<0.001$). At baseline, 12 weeks, 24 weeks and 48 weeks after treatment were significantly different in the levels of ALT, AST, TBIL, HBVDNA (all $p$ less than 0.05). HBV viral load dropped sharply during the first two weeks. Along with the extension of treatment time, the clinical indexes gradually reduced. The rates of HBVDNA undetectable in 12 weeks, 24 weeks and 48 weeks after treatment were accounted for 32.4% (44/136), 46.3% (63/136) and 78.7% (107/136), respectively. The rates of HBeAg seroconversion were accounted for 2.9% (4/136), 3.7% (5/136) and 14.7% (20/136), respectively. Compared with pre-therapy level, a significant decrease in serum levels of IL-17, TGF-β, IL-10, IL-23 and RORγt and Foxp3 expression were found from week 48 ($p<0.001$).

**Conclusion:** Antiviral therapy with entecavir can improve liver function, inhibit virus replication and lower the levels of the cytokines of Th17 and Treg, and control the progress of liver disease.
Flavonoid glycosides from Japanese Camellia oil cakes and their inhibitory activity against advanced glycation end-products formation

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Background: The Japanese Camellia (Camellia japonica L.; Japanese name: Tsubaki) is one of the most well-known species in the genus Camellia, Theaceae family. The seeds are used as oil materials in food and cosmetics industries. Oil cakes were obtained as by-products during seed oil production, and most of them were unutilized. Advanced glycation end-products (AGEs) were produced from the carbonyl groups of the reducing sugars reacting with the free amino groups of proteins. Inhibition of AGEs formation has been paid attention as a potential therapy for many aging-related diseases. In this study, we reported the isolation and structural elucidation of flavonoid glycosides from oil cake of C. japonica seeds, as well as their AGEs formation inhibitory activities.

Methods: The oil cakes were extracted with hot water. Isolation and separation was carried out by column chromatography and preparative HPLC. Structural elucidation was achieved by analysis of spectroscopic data, including 1D and 2D NMR, and MS. Chemical profiling of the oil cake extract was characterized by HPLC-PDA analysis. Anti-AGEs activity was carried out by D-ribose-BSA assay and GA/MGO/GO-HSA assay.

Results: Phytochemical investigation resulted in the isolation and structural elucidation of thirteen flavonoid glycosides, including four novel compounds. The extract and isolated compounds exhibited potent inhibitory activities against AGEs formation. The extract and the major compound 7 also inhibited AGEs formation between HSA and three intermediates, GA/GO/MGO. Moreover, extract and 7 decreased the GA-HSA enhanced expression of MIP-1β mRNA in differentiated U937 cells.

Conclusions: The extract and its flavonoid glycoside constituents from the oil cakes of C. japonica seeds possessed potential usability as health materials against AGE-associated chronic diseases.

Keywords: Flavonoid glycoside, Japanese Camellia, Advanced glycation end-products
S-VI-2

Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 promotes lipid accumulation and lipid droplet formation in the liver of nonalcoholic steatohepatitis (NASH) model mouse

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Nonalcoholic steatohepatitis (NASH) is characterized by lipid accumulation with inflammation and fibrosis in the liver. STARD10 is a member of the START domain-containing lipid transfer protein family. We have previously shown that STARD10 is highly expressed in the liver. When fed a high fat diet, Stard10 knockout (Stard10−/−) mice accumulated significantly less cholesterol and triglycerides (TG) in the liver than wild type (WT) mice. The purpose of this study was to clarify the role of STARD10 in lipid accumulation associated with NASH in the liver. We examined the effect of STARD10 disruption on lipid accumulation in the liver of NASH model mice that were induced by choline-deficient L-amino acid-defined diet (CDAA). Stard10−/− mice fed CDAA gained weight and epididymal fat in a manner similar to WT mice. However, the liver of Stard10−/− mice was smaller in size and the area of lipid droplet (LD) in hepatocytes of Stard10−/− mice was significantly smaller than those of WT mice. Gene expression levels of proinflammatory cytokines and fibrosis marker genes were significantly lower in the liver of Stard10−/− mice compared with WT mice, suggesting that STARD10 regulates these genes through the promotion of lipid accumulation. Lysophosphatidylcholine acyltransferase 1 (LPCAT1) is an enzyme which catalyzes the conversion of lysophosphatidylcholine to phosphatidylcholine (PC). We found that the STARD10 plays a role in promoting LD formation through the interaction with LPCAT1. We confirmed the colocalization of STARD10 and LPCAT1 at the LD membrane and their interaction. In conclusion, our study indicated that STARD10 is involved in fine-tuning the balance between PC and TG to promote LD formation in the liver through the interaction with LPCAT1.

Keywords: NASH, lipid transfer protein, lipid droplet
S-VI-3

From curcumin to its derivatives for multi-drug resistance leukemia treatment

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Resistance to chemotherapeutic drugs is a major cause of death in many cases of cancer patients. Multi-drug resistant (MDR) phenotypes are characterized by their simultaneous resistance against different drugs that have no structural similarities. Increasing the extracellular efflux of chemotherapy drugs by ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp) is one of a known mechanism of MDR. In this study, nine curcumin derivatives were synthesized and their multi-drug resistance (MDR) reversing properties were determined in human MDR leukemic (K562/Adr) cells. Two groups of the curcumin derivatives were used in this study, group 1 was diketone and group 2 was cyclohexanone derivatives, respectively. From cytotoxicity testing found that four derivatives including compounds 1J, 2A, 2F and 2J could significantly improve the sensitivity of K562/Adr cells to paclitaxel (PTX) for 8-, 2-, 8- and 16- folds, respectively. The mechanism in increasing in drug sensitivity of the multi-drug resistance leukemia was further investigated and found that compound 1J could inhibit P-gp function, while 2A and 2F could inhibit P-gp expression. Interestingly, compound 2J exerts inhibition of both P-gp function and expression. The combination between 2J and PTX in K562/Adr cells indicated strong synergistic effects, which likely due to its MDR reversing activity. Moreover, 2J showed less cytotoxicity to human normal cells and red blood cells suggesting the safety of the compound.

Keywords: Curcumin derivatives, Multi-drug resistance, Leukemia
S-VI-4
Quantification of repolarization reserve for the risk prediction of drug-induced arrhythmia

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A number of drugs under development and in clinical use turned out to induce arrhythmia and were withdrawn from the market. Drug-induced arrhythmia is mainly accounted for by block of one type of repolarizing K⁺ currents in cardiac myocyte called \( I_{Kr} \). However, the risk of drug-induced arrhythmia differs among various \( I_{Kr} \) blockers because normal repolarization of membrane potential in myocyte is determined by multiple and redundant mechanisms. Currents other than \( I_{Kr} \) can compensate the blocked \( I_{Kr} \). This compensation for repolarization is called repolarization reserve. The vaguely defined concept was used only for a qualitative explanation to account for different risks among drugs. Aiming at utilizing the concept of repolarization reserve for the accurate prediction of drug-induced arrhythmia, in the present study, we developed a method for quantification of repolarization reserve. We redefined repolarization reserve as a source of repolarization currents that are activated during prolonged depolarization. By using human ventricular myocyte simulation models, we searched a novel quantification method based on this definition. We calculated a new quantitative index of repolarization reserve by simulating action potential (AP) clamp with prolonged AP waveforms and the human ventricular myocyte models. The calculated index quantitatively accounted for prolongation of action potential duration (APD) under various conditions in cardiac myocytes. We also confirmed that the quantified repolarization reserve could be used to predict the prolongation of APD by different drugs. The proposed index for repolarization reserve is expected to contribute to further understanding of APD prolongation. Therefore, our method may contribute to efficient developments of safe drugs by predicting the risk of drug-induced arrhythmia.

Keywords: drug-induced arrhythmia, simulation, repolarization reserve
S-VI-5
Development of a High-Throughput Screening Paradigm for the Discovery of Small-Molecule Agonists on Adenylyl Cyclase 2 Isoform

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Background: The Adenylyl Cyclase (ACs) isoforms are the important target for the drug discovery of heart failure, COPD, and Alzheimer’s disease. AC2 is potentially involved in lung diseases, small molecules that selectively regulate the enzyme will be potential drugs. This study was to establish a high-throughput screening system as AC2 target, to find more novel AC2 agonist, which could provide the basis for the development of new drugs for the treatment of chronic obstructive pulmonary disease (COPD).

Methods: We transfected mammalian cells HEK293 stably expressing hAC2. The cellular cAMP levels were measured using time-resolved fluorescence resonance spectrometry (TR-FRET) in order to evaluate the effects of compounds on enzyme activity. Candidate compounds from: 1. FSK derivatives isolated from Coleus forskohlii --- Yunnan special medicinal plant; 2. Based on AC2 crystal structure, through virtual screening, structure similarity search of compound libraries, forming the corresponding ligand library.

Results: 1. Z factor of enzyme activity test system is in the range of 1>Z>0.5 and no significant fluctuations, showing good stability of the drug screening system. 2. The dose-effect evaluation of FSK and its derivatives: EC50 of FSK, AG-690, FSK/D1 and ISOF/D2 are 0.84 (uM), 3.72(uM), 2.98(uM), and 2.63 (uM), respectively. 3. Candidate compounds in the ligand library were evaluated by the cell screening system: KM-B&M08, EC50=1.85 (uM).

Conclusion: This study combined with the traditional chemical derivatization and computer-assisted molecular design, we successfully established a high-throughput screening paradigm for the discovery of small molecule agonists of AC2 isoform. The screening effort identified five active compounds, having dose related, to lay good foundation for the further development of anti-COPD drugs.

Keyword: Adenylyl Cyclase, virtual screening, COPD
Enhancing student learning with interactive teaching methods

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**Background:** The purposes of this session are to inform participants of terms, definitions, underpinning theories and applications of active learning.

**Methods:** This is an interactive lecture. The activities in this workshop provide participants with examples of interactive techniques, which can be used in classrooms. The activities give opportunities for participants to engage with the content in the workshop.

**Results:** Active Learning Classrooms are spaces designed to support teaching and learning in an atmosphere that contributes to engaging students actively in their own learning. The principles of effective design involve student-faculty interaction, active and collaborative learning, enriching educational experiences, and a supportive campus environment.

**Conclusion:** The student learning experience can be enhanced by incorporating active learning into the curriculum and transforming the classroom into an exciting, dynamic learning environment.

**Keywords:** active learning, interactive teaching methods, medical education
S-VII-2
Active learning in Toho University Faculty of Medicine

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Background: It is said that medical education in Japan is biased toward mastery of knowledge and education on skills and attitudes is inadequate. After the ECFMG statement in 2010, we have transformed from process-based education to outcome-based education that clearly shows the learning objectives. Clinical practice has improved both quality and quantity, and also in pre-clinical practice education, students are required to study memorization (knowledge) themselves. In the classes, it is required to think themselves, discuss and present themselves based on that knowledge. That is, gradually but active learning on skills and attitudes has come to be carried out.

Contents: Even at Toho University, some lessons are focused on students thinking, discussing, and presenting themselves rather than acquiring knowledge. Active learning classes are being developed in humanity education (whole-human medical personnel education), which are being conducted from 1st to 6th year, integrated social medicine practice and clinical reasoning PBL tutorial classes. In this presentation, we will introduce the active learning class in our university.

Conclusion: Active learning is progressing not only in our university but also in medical school in Japan. Further improvement is also required at our university so as not to miss the trend of medical education of the world.

Keywords: Active learning, PBL tutorial, Humanity education
The efficacy of Mesenchymal stem cells (MSCs) and Platelet rich plasma (PRP) against partial thickness cartilage defect (PTCD)

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Background: PTCD will not be repaired because of not induced bone marrow stem cell. However some previous paper reported spontaneous repair of PTCD in young animal. And the detail of age related response against PTCD in animal including rat is still unclear. So our purpose is to assess the detail of age related response against PTCD using each age rats and the efficacy of MSCs and PRP against PTCD using age-dependent repair natural course as a reference.

Methods: Firstly, 3, 6, 10, 14 week old Sprague Dawley (SD) rats were used. PTCD was made at the weight bearing part of medial femoral condyle by 100μm converted ophthalmic knife. The knees were harvested at 1, 7, 14, 28, 84 days after PTCD and histology was assessed by Safranin O staining. Secondly, intervention materials were PBS, PRP, MSCs only, MSCs and PRP against 14 week-old rats. We used infra-patella fat pad derived MSCs and injected one million MSCs. Histological evaluation was performed at 4 weeks after the treatment. As histological evaluation, we used age-dependent repair natural course as a reference.

Results: Excluding 14 week-old rats, scores totally tended to improve. And at 4 weeks and 12 weeks after PTCD, difference between each age group was similar and, then only 10 week-old and 14 week-old were not significantly different. In 6 week-old rats and under the age, PTCD can be repaired. As assessing interventions, MSCs only, and MSCs and PRP groups were significantly better than PBS and PRP group regarding histological evaluation.

Conclusion: PTCD can be repaired in 3 and 6 week-old rats. MSCs were effective to repair PTCD.

Keywords: Mesenchymal stem cells, Platelet rich plasma, Partial thickness cartilage defect
A Practical Green Extraction and Standardization Method for Development of Phytomedicines

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Background: Mangosteen pericarps are a by-product of the mangosteen juice factory. The pericarp extracts are enriched in xanthones, particularly α-mangostin, which has been used as a marker for their standardization. Extracts from mangosteen pericarps have been reported to have various pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and wound healing properties, and have therefore been used in several commercial pharmaceutical products and cosmetics. It has been reported that dichloromethane was the most suitable solvent for extraction of α-mangostin. However, the use of dichloromethane as a solvent for extraction is restricted due to its toxicities. The absence of risk during extraction and the safety of the ingredients used are a major concern and have drawn attention towards the need to use a greener solvent.

Methods: The present studies have focused on investigating the use of excipients for topical formulation that has including isopropyl myristate and cetyl alcohol as alternative green solvents for extraction using a microwave assisted green extraction method. These solvents have similar polarities to dichloromethane, but they are considered to be safer and cheaper. The content of α-mangostin was determined by an HPLC method.

Results: The obtained extracts contained α-mangostin of not less than 2.0% w/w, and can be used directly for topical formulations without the step of solvent evaporation, and have resulted in a reduced cost of production. The medicinal creams and gels containing of 0.1-0.2% w/w α-mangostin were then formulated using these extracts.

Conclusion: This green extraction and standardization method for the extracts from mangosteen pericarps may be a practical approach for development of their pharmaceutical products and cosmetics.

Keywords: Garcinia mangostana, α-mangostin, xanthone, green extraction
In Vivo Proof of Concept of Adoptive Immunotherapy for Hepatocellular Carcinoma Using Allogeneic Suicide Gene-Modified Killer Cells

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Background: Cell therapy based on alloreactivity has completed clinical proof of concept against hematological malignancies. However, the efficacy of alloreactivity as a therapeutic approach to treat solid tumors is unknown. Using cell culture and animal models, we aimed to investigate the efficacy and safety of allogeneic suicide gene-modified killer cells as a cell-based therapy for hepatocellular carcinoma (HCC), for which treatment options are limited.

Methods: Allogeneic killer cells from healthy donors were isolated, expanded, and phenotypically characterized. Antitumor cytotoxic activity and safety were studied using a panel of human or murine HCC cell lines engrafted in immunodeficient or immunocompetent mouse models.

Results: Human allogeneic suicide gene-modified killer cells (aSGMKCs) exhibit a high, rapid, interleukin-2-dependent, and non-major histocompatibility complex class I-restricted in vitro cytotoxicity toward human hepatoma cells, mainly mediated by natural killer (NK) and NK-like T cells. In vivo evaluation of this cell therapy product demonstrates a marked, rapid, and sustained regression of HCC. Preferential liver homing of effector cells contributed to its marked efficacy. Calcineurin inhibitors allowed preventing rejection of allogeneic lymphocytes by the host immune system without impairing their antitumor activity.

Conclusion: Our results demonstrate proof of concept for aSGMKCs as immunotherapy for HCC and open perspectives for the clinical development of this approach.

Keywords: Adoptive Immunotherapy, Suicide Gene, Hepatocellular Carcinoma
P-1
Pathogenic Role of Resistin in Rheumatoid Arthritis

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Background: Adipose tissue synthesizes and releases physiologically active molecules that are known as adipokines. Resistin, an adipokine, has been widely studied the regulation of glucose homeostasis and insulin sensitivity. Moreover, resistin also plays an important role of inflammation. We previously reported that serum level of resistin correlated with the disease activity of rheumatoid arthritis (RA). However, the pathogenic role of resistin has not been elucidated. In this study, we examined the stimulatory effect of resistin on fibroblast-like synoviocytes (FLSs) form RA patients.

Methods: Expression of resistin and the receptor, adenylyl cyclase-associated protein 1 (CAP1), in the synovial tissue from RA and osteoarthritis (OA) was examined by immunohistochemistry. FLSs were incubated with resistin for 18 hours. Then, total RNA was extracted, and the gene expression profile was analyzed by RNA sequencing. Concentration of chemokines in the culture supernatant was determined by enzyme-linked immunosorbent assay (ELISA). Expression of CAP1 was examined by RT-PCR and Western blotting. To verify signaling of resistin via CAP1, we transfected siRNA for CAP1 before stimulation with resistin.

Results: Resistin and CAP1 was abundantly expressed in the RA synovial tissue. Resistin expression was minimal in the OA synovium. Double immunofluorescence staining revealed that CD68-positive macrophages expressed resistin in RA synovium. CAP1 was expressed by cadherin-11-positive FLSs in RA. RT-PCR and Western blotting showed that in vitro cultured FLSs also expressed CAP1. RNA sequencing revealed that expressions of 18 genes, including 7 chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8 and CCL2), from RA FLSs were increased more than 2 folds by stimulation with resistin. Production of CXCL8 and CCL2 in the culture supernatant of FLSs was increased by resistin. Transfection with CAP1 siRNA suppressed resistin-induced CXCL8 production by FLSs.

Conclusion: Resistin might play an important role in the pathogenesis of RA via upregulation of chemokine production in the synovial tissue.

Keywords: Resistin, Chemokine, Rheumatoid arthritis
SATB1 deficiency in mice leads to Sjögren’s syndrome-like autoimmune exocrinopathy

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Sjögren’s syndrome (SS) is a chronic autoimmune disease in which the patient’s lymphocytes infiltrate and destroy the lachrymal and salivary glands. However, the molecular mechanism of SS pathogenesis is poorly understood. SATB1 (Special AT-rich sequence binding protein-1) is a genome organizer that regulates chromatin structure and gene expression. SATB1 conditional knockout (SATB1cKO) mice, in which SATB1 gene is specifically deleted in hematopoietic cells are autoimmune prone with age caused at least in part by defective thymic central tolerance (Kondo M., Tanaka Y. et al, J. Immunol. 196: 563-572, 2016). Furthermore, we uncovered SS-like symptoms developed in young SATB1cKO mice.

In this study, we analyzed the detail of the SS-like symptoms in SATB1cKO mice. We found that mononuclear cell infiltration into salivary gland and production of saliva was significantly lower even at young age (4 weeks) in SATB1cKO mice. Female mice manifested with an earlier onset of the disease than male mice, suggesting that female mice are more susceptible to SS. Interestingly, these young SATB1cKO mice did not produce some autoantibodies which are increasing in elder SATB1cKO mice and in patient with SS. In addition, we did not observe mononuclear cell infiltration into other organs and immune-complex-mediated glomerulonephritis in young SATB1cKO mice. These results indicate that young SATB1cKO mice may be useful as a new animal model for the SS. We discuss the scheme of autoimmune disease progression especially the early stage of SS.

Keyword: SATB1, Sjögren’s syndrome, autoimmune exocrinopathy
P-3

Targeting FOXM1 in triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) represents an important clinical challenge, as it does not respond to conventional endocrine therapies or other available targeted agents. Oncogenic transcription factor FOXM1 has been shown to be upregulated in the majority of human cancers, including breast cancer. We have previously reported that FOXM1 is a key player in tumour growth as well as drug resistance leading to poor survival in breast cancer patients. However, the anti-metastatic effect of FOXM1 inhibition as well as the mechanisms by which FOXM1 regulates breast cancer TNBC metastasis remain to be elucidated.

Methods: In this study, we inhibited FOXM1 using the FOXM1 inhibitors Thiostrepton and FDI-6 in MDA-MB-231 and MDA-MB-436 cell lines. The anti-cancer ability of FOXM1 inhibition to suppress growth and metastasis in TNBCs was then evaluated using 2D cell culture assays—SRB, wound healing and transwell migration assays—together with 3D tumour spheroid models.

Results: The results of the 2D cell culture assays showed that FOXM1 inhibition using either Thiostrepton or FDI-6 significantly reduced the long-term clonogenic growth as well as motility of TNBC cells. Thus, the inhibition of FOXM1 expression and activity could be an attractive option for treating TNBC. However, we are still in the process of result validation using more physiological cellular systems and 3D tumour spheroid models. In addition, the molecular mechanisms related to breast cancer cell migration and invasion as well as their clinical significance are also the focus of future investigation.

Conclusion: Targeting FOXM1 expression and activity could be a promising strategy for treating TNBC. We expect that the results of this study, together with the development of more specific inhibitors as well as delivery systems, will assist in opening up an effective therapeutic window where targeting FOXM1 could achieve clinical benefits.

Keywords: FOXM1, metastasis, triple-negative breast cancer
P-4

Down-Regulation of 14-3-3γ to suppress migration and invasion of lung adenocarcinoma cells by inhibiting epithelial-mesenchymal transition

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Background: The 14-3-3γ is involved in the tumorigenesis and metastasis of lung cancer. However, functional roles regarding tumor cell invasion and their underlying mechanisms in NSCLC are still incomprehensible. We aimed to evaluate the effect of 14-3-3γ down-regulation on cell migration in A549 and H358 human lung cancer cell lines.

Methods: Cells were transfected with small interfering RNA (siRNA) targeting the 14-3-3γ gene. The expression levels of 14-3-3γ, MMP-2/-9, and invasion-regulated proteins in the epithelial-mesenchymal transition (EMT) pathway following the 14-3-3γ-siRNA transfection were detected by western blot analysis. The activity of MMP-2/-9 was determined by gelatin zymography, and both cellular migration and invasion were analyzed using Transwell assay. Statistical significance was determined via Student’s independent t-test by comparison to control groups. A P-value < 0.05 was considered statistically significant.

Results: A decreased 14-3-3γ expression in A549 cells led to a significant inhibition in the migration and invasion—approximately 39% and 59%, respectively—compared to controls (P < 0.001) as well as a significant reduction in migration and invasion in H358 cells, which were found to be 62% (P = 0.001) and 65% (P = 0.003), respectively. In addition, we found that the expression levels of vimentin in snails and slugs were significantly reduced—approximately 30%-60%—in the 14-3-3γ-siRNA group compared with those of the control groups in both A549 and H358 cells.

Conclusion: Our results suggested that the down-regulation of 14-3-3γ inhibits NSCLC cell migration and invasion by suppressing the EMT pathway.

Keywords: 14-3-3γ, migration, invasion, siRNA, lung adenocarcinoma cell
P-5

The role of WT1 isoforms in vasculogenic mimicry and metastatic potential of human triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) is highly aggressive and has few therapeutic treatments. The new targeted therapy and biomarkers provide an alternative choice. TNBC overexpresses many candidate molecules that play a role in the progression of cancer. Previous studies have found a high expression of WT1 in TNBC. It has 4 major distinct isoforms; isoform A (–17AA/–KTS; –/–), isoform B (+17AA/–KTS; +/–), isoform C (–17AA/+KTS; –/+), and isoform D (+17AA/+KTS; +/+). However, their molecular mechanisms involved in TNBC progression remains largely unclear.

Methods: MDA-MB-231 was stably transfected with WT1 isoforms and the aggressiveness behaviors were evaluated using wound healing, migration, invasion and tube formation assay. The molecular mechanism of the involved genes and proteins were investigated by qRT-PCR and western blot analysis, respectively.

Results: An increased number of vasculogenic mimicry (VM) formations was observed in MDA-MB-231 transfected with WT1 isoform B and C. VM formation in the tube formation assay depends on a special process of invasion and migration of cancer cells. Our results indicated that MDA-MB-231/WT1 isoform B and C enhanced cell migration in vitro. Moreover, MDA-MB-231/WT1 isoform B showed the highest rate of cell invasion compared to other isoforms that may be involved in VM formation. Finally, compared to parental cells and other isoforms, MDA-MB-231/WT1 isoform B exhibited the highest rate of β-catenin and vimentin expression. Several studies have suggested that the Wnt/β-catenin signal pathway is involved in VM formation and progression.

Conclusion: Our results demonstrated that WT1 isoform B promoted a more aggressive phenotype in MDA-MB-231 cells than other isoforms through the β-catenin and vimentin expression leading to VM and invasion induction. These phenotypes may play critical roles in increasing aggressiveness and facilitating metastasis in TNBC.

Keywords: WT1, vasculogenic mimicry, β-catenin, MDA-MB-231
Low concentrations of Mupirocin Promote Methicillin-Resistant *Staphylococcus aureus* USA300 Clone’s Biofilm Formation

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**Background:** The objective of this study was to investigate the impact of mupirocin against superficial skin infections. This was compared to other classes of antibiotics such as cefazolin, levofloxacin, gentamicin and erythromycin on planktonic cell growth and biofilm formation of *Staphylococcus aureus*.

**Methods:** The minimum inhibitory concentration (MIC) has been determined by broth microdilution. Crystal violet binding assay was used to investigate the biofilm formation. Biofilms structure and viability of bacteria has been observed by fluorescent confocal laser scanning microscope (CLSM), and biofilm-related genes expression has been quantified by real-time polymerase chain reaction (RT-PCR).

**Results:** MRSA USA300 clone was resistant to mupirocin, whereas MRSA ATCC-43300 and MSSA ATCC-29213 were susceptible. The pattern of mupirocin on biofilm formation exhibited a biphasic dose-response curve characterized by low-dose stimulation and high-dose inhibition. CLSM images showed a spontaneous mat-like biofilms established with a higher in cells density in mupirocin treated group. The expression of RNAIII both planktonic cell and biofilm was the dominant gene, whereas *agrA* and *sarA* were not different. However, the genes expression by other classes of antibiotics showed fluctuations at low level expression.

**Conclusion:** The present study revealed low concentrations of mupirocin promoted biofilm formation of MSSA and MRSA, particularly in MRSA USA300 clone.

**Keywords:** mupirocin, antibiotics, biofilms formation, MRSA USA300
Biomimetic-like nanoparticles for sepsis management

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Background: Sepsis resulting in uncontrolled inflammatory responses to bacterial infections continues to cause high rates of morbidity and mortality worldwide. Currently, effective sepsis treatment is unavailable in clinics and care remains primarily supportive. Herein, we report the development of biomimetic nanoparticles that mimic macrophage targets for the management of sepsis.

Methods: The nanoparticles made by wrapping polymeric cores with a cell membrane derived from macrophages posses an antigenic exterior similar to that of source macrophage cells.

Results: By acting as macrophage decoys, these nanoparticles bind and neutralize endotoxins that otherwise trigger immune activation. In addition, these macrophage-like nanoparticles are capable of binding with pro-inflammatory cytokines and, hence, inhibit their bioactivities in potentiating the sepsis cascade. Using a mouse bacteremia model, we demonstrated that treatment with macrophage mimicking nanoparticles reduced pro-inflammatory cytokine levels, inhibited bacterial colonization, and eventually conferred a higher survival rate for the infected mice.

Conclusion: Overall, MΦ-NPs, as a new biomimetic detoxification strategy, show some promise in the improvement of the clinical outcome of sepsis management, and potentially shift the current paradigm of the clinical detoxification therapy.

Keywords: macrophage, biomimetic nanoparticle, sepsis, lipopolysaccharide, proinflammatory cytokines.
Poster

**P-8**

**Extent of Bacterial Contamination of Electronic Warm Water Bidet Toilets in a University Hospital Setting**

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**Background:** Bidet toilet seats feature electric toilet seats with a nozzle spraying warm water to the genitalia, anal, and perianal area. In recent years, installation of bidet toilets within hospitals in Japan has raised concerns regarding potential for cross-contamination by antimicrobial resistant bacteria from patients who are hospitalized over an extended period.

**Methods:** All 292 electronic bidet toilets at a university hospital were sampled for contamination. Swabs for culture were used to sample water-jet nozzles, and seats. Following incubation, colonies were identified, and antimicrobial susceptibility testing was performed. Identification of antimicrobial resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), and extended-spectrum β-lactamase (ESBL) producers was performed. Pulsed field gel electrophoresis (PFGE) was also performed on the isolates.

**Results:** Of the 292 bidet toilet seats sampled, warm water nozzles of 254 (86.9%) were found to be contaminated by one or more of the following organisms: *S. aureus*, *Streptococcus* sp., *Enterococcus* sp., Enterobacteriaceae, and non-Enterobacteriaceae gram-negative bacteria. *S. aureus* was recovered from one water-jet nozzle and 9 toilet seats of which MRSA was found in a water-jet nozzle, and a toilet seat. Both nozzle and seat of the same toilet were contaminated with a CTX-M-9 group ESBL-producing *Escherichia coli* with an identical PFGE band pattern. *Pseudomonas aeruginosa* was isolated from 6 water-jet nozzles, and *Acinetobacter* species was isolated from 7 water-jet nozzles, and toilet seat. Of the gram-negative isolates recovered from samples, the organism with the highest frequency of isolation was *Stenotrophomonas maltophilia* which was recovered from 39 toilets.

**Conclusion:** Warm-water nozzles of bidet toilets are contaminated with a wide range of bacteria making it a potential vehicle of cross-infection. In the hospital setting, shared use of bidet toilets must consider the clinical background of the patient. Based on our findings, these devices must be part of the risk management program and include steps for monitoring and disinfection.

**Keywords:** Electronic bidet toilets, Antimicrobial resistant bacteria, Bacterial contamination
Electropharmacological effects of antibiotic azithromycin in microminipigs: Propose of new potential mechanisms for lethal arrhythmia leading to sudden death

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Background: Azithromycin has been reported to increase the cardiovascular risk and sudden cardiac death in the patients with high baseline risk, which raised concerns for its arrhythmogenic potential. Although azithromycin suppresses $I_{Na}$, $I_{Kr}$, $I_{Ks}$, $I_{CaL}$ and $I_{K1}$ in the heart, its electropharmacological effect leading to the onset of lethal arrhythmias is not fully investigated.

Methods: Using an extraordinary small size of miniature pigs; microminipigs, weighing approximately 10 kg (male, n=4), we examined electropharmacological effects of azithromycin in doses of 0.3, 3 and 30 mg/kg under the halothane anesthesia, which were intravenously infused over 10 min with an interval of 20 min.

Results: The low dose did not alter any of the cardiovascular variables. The middle dose significantly shortened the QT interval and QTc for 10-30 min, whereas it tended to increase the heart rate for 10-20 min. The high dose significantly decreased the mean blood pressure for 5-15 min, prolonged the QRS width at 15 min but shortened the QT interval at 15 min, whereas it tended to increase the heart rate for 5-15 min.

Conclusion: Prolongation of the QRS width by the high dose indicates that azithromycin may suppress ventricular $I_{Na}$ in vivo, which may unmask Brugada electrocardiogaphic genotype in susceptible patients. Meanwhile, abbreviation of the QTc by the middle dose might cause potentially lethal, short QT-related, cardiac arrhythmia syndrome.

Keywords: Azithromycin, Microminipigs, Short QT syndrome, Brugada syndrome
JNK signaling dynamics controls cytokine expressions

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JNK (c-jun N-terminal kinase) plays key roles in inflammatory response. Therefore dysregulation of JNK is known to cause neurodegenerative diseases or malignant transformation of cells. Previous studies reported that transient JNK activation causes survival response, while sustained JNK activation is responsible for apoptosis. However, how JNK signaling controls multiple cellular functions is unclear because information of JNK activity in live cells is missing. Here, we focused on the relationship between JNK signaling dynamics and downstream gene expression. To understand JNK signaling dynamics, we visualized JNK activity in HeLa cells by using a novel FRET reporter. We then applied repetitive IL-1β (10 ng/ml) stimulation to the cells to analyze the regulation of JNK activity.

In the repetitive IL-1β stimulation experiment, the amplitude of JNK activity became higher when stimulation pulses were given at low frequency, but not at high frequency. To understand the relationship between JNK dynamics and downstream gene expressions, we determined IL-6 and IL-8 mRNA expressions by periodic IL-1β stimulation. JNK activity could be varied depending on the temporal patterns of IL-1β stimulation. IL-1β-dependent activation of IL-6 and IL-8 were suppressed in the presence of JNK inhibitor SP600125 (20μM). When the expression of IL-6 and IL-8 were induced by repetitive IL-1β pulse stimulation, the mRNA expression levels were increased in proportional to the frequency but not to the total exposure time of the IL-1β stimulation. Our data clearly indicate that JNK is dynamically regulated in living cells, and the temporal pattern of JNK activity determines downstream cytokine expression.

Keywords: JNK, FRET, dynamics
Amitriptyline may have possibility to induce Brugada syndrome rather than long QT syndrome

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Background: Amitriptyline is a tricyclic antidepressant that is known to induce long QT syndrome; however, its potential to induce Brugada syndrome is less studied. We qualitatively and quantitatively analyzed the potential of amitriptyline to induce these lethal syndromes by using the halothane-anesthetized dogs (n=6).

Methods: Amitriptyline was intravenously administered in doses of 0.1, 1 and 10 mg/kg over 10 min with 20 min interval between each dose, which would provide approximately 1, 10 and 100 times higher plasma concentrations than a therapeutic one, respectively.

Results: The low dose hardly altered any of the cardiovascular variables. The middle dose increased the heart rate, cardiac output and left ventricular contractility, but decreased the total peripheral vascular resistance and left ventricular end-diastolic pressure, whereas it did not alter any of the electrocardiographic variables. The high dose decreased the mean blood pressure and left ventricular contractility; suppressed atrioventricular nodal and intraventricular conduction; shortened the repolarization period without altering the J-Tpeakc and Tpeak-Tend, and prolonged the effective refractory period, providing post-repolarization refractoriness in addition to the enhancement of the middle dose-induced cardiovascular effects.

Conclusion: Amitriptyline at up to 100 times its therapeutic concentration may not be associated with the onset of long QT syndrome, but may induce Brugada syndrome.

Keywords: Amitriptyline, Long QT syndrome, Brugada syndrome
A novel drug candidate for atrial fibrillation: Cardiovascular effects of M201-A in canine models

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Background: Most of antiarrhythmic drugs for atrial fibrillation alter the electrophysiological profile of both atrium and ventricle. M201-A is a 1,4-benzothiazepine derivative that inhibits both $I_{K,ACh}$ and $I_{Kur}$ which were known to be selectively expressed in atrium. In this study, we simultaneously assessed the pharmacological and adverse effects of M201-A using 2 types of in vivo canine models.

Methods: [Exp. 1] We administered M201-A in doses of 0.03, 0.3 and 3 mg/kg i.v. over 10 min to the halothane-anesthetized dogs to evaluate its cardiohemodynamic and electrophysiological effects (n=4). [Exp. 2] We administered M201-A in doses of 1 and 3 mg/kg i.v. over 10 min to the chronic atrioventricular block dogs to assess its torsadogenic potential (n=4).

Results: [Exp. 1] The low dose did not alter any of the cardiovascular variables. The middle dose prolonged the QTc without altering the other variables. The high dose increased the mean blood pressure, shortened the AH interval and prolonged the QTc and atrial effective refractory period without altering the other variables including the ventricular effective refractory period. [Exp. 2] Both doses prolonged the QTc, but did not alter QT interval, short-term variability of repolarization or the number of ventricular contractions. No torsade de pointes was detected during the experimental period.

Conclusion: These results indicate that M201-A exhibits preferable pharmacological characters, i.e. high atrial specificity and lack of proarrhythmic potential, as a novel drug candidate for atrial fibrillation. Further studies using persistent atrial fibrillation model would be helpful to test its efficacy against atrial fibrillation.

Keywords: atrial fibrillation, drug candidate, in vivo canine model
P-13

Effects of ethyl acetate fraction from Senna garrettiana heartwood on chronic inflammation in vivo

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Background: Senna garrettiana (S. garrettiana), a Thai medicinal plant in Caesalpiniaceae family, is locally known in Thai as “Samae-sarn”. The heart wood of this plant has been used to treat pain and feminine diseases. Phytochemical investigations of S. garrettiana indicate the presence of different chemicals especially piceatannol, a phenolic stilbene derivative that has a wide spectrum of biological activities including anti-inflammatory activity in vitro. Previous study of ethyl acetate fraction from heartwood of this plant showed a prominent antinociceptive activity. In the present study, the anti-inflammatory activities of ethyl acetate fraction from S. garrettiana heartwood were evaluated in rodent models.

Methods: The methanol extract of S. garrettiana heartwood was prepared and then a fractionation process was performed to obtain ethyl acetate fraction. Effects of ethyl acetate fraction (50, 100 and 200 mg/kg) on chronic inflammation were investigated using cotton pellet-induced granuloma and complete Freund’s adjuvant-induced arthritis in rats.

Results: Oral administration of ethyl acetate fraction (50, 100 and 200 mg/kg) dose-dependently decreased the granuloma weight with the percent inhibition of 11.41, 15.92, and 26.13, respectively. Ethyl acetate fraction suppressed the edema in arthritic rats on day 7, 10, and 13 in a dose-dependent manner. At the dose of 200 mg/kg, it attenuated the swelling at day 13 with a reduction of 63.27%.

Conclusion: These results demonstrated that ethyl acetate fraction of S. garrettiana heartwood possess anti-inflammatory activities and its actions might be at least attributed to modulation of inflammatory mediators.

Keyword: S. garrettiana heartwood, granuloma, arthritis, inflammation.
Pharmacological effects of Mongolian medicinal plant Adonis mongolica on cardiovascular system

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Background: Adonis mongolica (Ranunculaceae) is one of endemic plants in Mongolia and has been used as a medicinal herb in Mongolian traditional medicine to treat patients with congestive heart failure showing tachycardia and edema. Although the plant has been empirically used, the precise information regarding its cardiovascular profile is still limited.

Methods: We assessed the cardiohemodynamic and electrophysiological profile of the water-soluble extract of Adonis mongolica using the guinea-pig in vivo model (n=4) and in vitro preparation (n=4-17). In addition, the onset mechanism of the extract-induced effects on the heart was pharmacologically analyzed (n=4-5).

Results: The extract exerted the positive chronotropic and inotropic, negative dromotropic, and vasopressor effects in addition to the proarrhythmic action, which were similar to the cardiovascular profile of cardiac glycosides. Also, liquid chromatography-mass spectrometry analysis showed that water-soluble extract of Adonis mongolica contained eight kinds of cardiac glycosides.

Conclusions: These results indicated that cardiac glycosides in the water-soluble extract of Adonis mongolica may explain currently observed various cardiovascular effects.

Keyword: cardiovascular, Adonis mongolica, Mongolian traditional medicine
P-15

Effects of 3,5,2',4'-tetrahydroxychalcone on serum urate and purine metabolism enzymes

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Background: To investigate the effects of 3,5,2',4'-tetrahydroxychalcone (P40) on the levels of serum uric acid in hyperuricemic rats and the purine metabolism enzymes in PC_{12} cells.

Methods: P40 (2.0, 4.0 and 8.0 mg/kg) was administrated intragastrically to rats twice daily for 5 times. The last dosage was given at 1 hour after an injection intraperitoneally with potassium oxonate (250 mg/kg) to increase the serum urate level. One hour later, blood and liver samples were collected from the mice after exposure to ethylcel ether for assay of urate. PC_{12} cells were cultured in DMEM containing 10% fetal calf serum with or without P40 and allopurinol, as positive control. After 48-hour exposure, the cells were harvested to extract RNA for determination of mRNA expression levels of hypoxanthine-guanine phosphoribosyl transferase (HGPRT), 5'-phosphoribosyl-1'-pyrophosphate synthetase (PRPS) and phosphoribosyl amidotransferase (PRPPAT) were measured by RT-PCR.

Result: P40 at a dose of 2.0 and 4.0 mg·kg^{-1} significantly reduced the serum urate levels, compared with untreated hyperuricemic rats induced by potassium oxonate ($P <0.01$). However, P40 has no influence on the hepatic urate levels. Moreover, P40 did not inhibit the mRNA expression of HGPRT, PRPS and PRPPAT in PC_{12} cell, at the concentration of $1\times10^{-8}$, $1\times10^{-7}$, $1\times10^{-6}$ mol/L.

Conclusion: P40 could reduce the uric acid level in hyperuricemic rats induced by potassium oxonate, but did not effect on the mRNA level of HGPRT, PRPS and PRPPAT in PC_{12} cell.

Keywords: 3,5,2',4'-tetrahydroxychalcone, hyperuricemia, Uric acid, HGPRT, PRPPAT, PRPS.

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Asian dust and pediatric emergency visits due to bronchial asthma and respiratory diseases in Nagasaki, Japan

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Background: The adverse health effects of Asian dust on the respiratory system of children are unclear. We hypothesized that Asian dust events may lead to increased visits by children to emergency medical centers due to bronchial asthma and respiratory diseases including bronchial asthma.

Methods: We used anonymized data on children receiving primary emergency treatment at Nagasaki Municipal Primary Emergency Medical Center, Japan between March 2010 and September 2013. We used Light Detection and Ranging (LIDAR) data to assess Asian dust exposure and performed time-stratified case-crossover analyses to examine the association between Asian dust exposure and emergency visits. The main analysis was done with data collected from March through May each year.

Results: The total number of emergency visits was 756 for bronchial asthma and 5,421 for respiratory diseases, and the number of “Asian dust days” was 47 during the study period. In school children, Asian dust events at lag3 and lag4 were associated with increased emergency visits due to bronchial asthma with odds ratios of 1.837 (95% Confidence Interval, 1.212-2.786) and 1.829 (95%CI, 1.179-2.806), respectively. Asian dust events at lag0, lag1 and lag2 were significantly associated with respiratory diseases among preschool children with odds ratios of 1.244 (95%CI, 1.128-1.373), 1.314 (95%CI, 1.189-1.452) and 1.273 (95%CI, 1.152-1.408), respectively. These associations were also significant when the results were adjusted for meteorological variables and other air pollutants.

Conclusions: The study findings suggested that Asian dust exposure increased emergency visits by children.

Keywords: Asian dust, Emergency visits, Children
Hepatitis B Screening Rates and Reactivation in Solid Organ Malignancy Patients Undergoing Chemotherapy in Southern Thailand

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Background: Hepatitis B virus reactivation (HBVr) following chemotherapy (CMT) is a well-known issue among hematologic malignancies, and recommended screening is well established. However, HBVr data in solid organ malignancy (SOM) patients is limited. This study aimed to determine HBsAg screening rates, hepatitis B virus (HBV) prevalence, and HBVr-related hepatitis incidence in SOM patients undergoing CMT in southern Thailand.

Methods: Based on the Oncology Unit’s registration database from 2009 to 2013, all SOM patients >18 years undergoing CMT at Songklanagarind Hospital, who were followed up until death or >6 months after CMT sessions, were retrospectively reviewed. The patients without baseline liver function tests (LFT) and who received CMT before the study period were excluded. The baseline clinical characteristics as well as the HBsAg screening and LFT data during follow-up were obtained and analyzed.

Results: Of the 3,231 cases in the database, 810 were eligible. The overall HBsAg screening rate in the 5-year period was 27.7%. The screening rates were low from 2009 to 2012 (7.8%-21%) and increased in 2013 to 82.9%. The prevalence of HBV among screened patients was 7.1%. Of those, 75% received prophylactic antiviral therapy. There were 3 HBVr-related hepatitis cases in this study; all of them were in the unscreened group.

Conclusion: The prevalence of HBV in SOM patients undergoing CMT in our study was similar to the estimated prevalence in the general Thai population. Interestingly, despite low overall HBV screening rates (27.7%), the incidence of HBVr-related hepatitis was very low. Therefore, HBV screening in all SOM patients undergoing CMT may not be warranted.

Keywords: hepatitis B virus, reactivation, chemotherapy, solid tumor, screening
P-18
Characteristics of outpatients with medically unexplained symptoms at a University Hospital, considering age

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Background: Diseases characterized by severe pain and other disorders exhibit so-called medically unexplained symptoms (MUS). The characteristics of patients with MUS are largely unexplained. In this presentation, the characteristics of a series of new patients with MUS, treated at a Japanese university hospital, are discussed.

Methods: Patients who visited the Department of Psychosomatic Medicine, Toho University, Omori Medical Center, between January and December of 2015, were enrolled. We evaluated the following, considering age as a factor; correlation between MUS and sex, academic background, chief complaints, reasons for visiting the medical center, diagnosis, symptoms, presence or absence of a referral form, continued treatment after the first visit, and marital status at the time of the respective examination.

Results: Of the patients studied, 70% displayed MUS; 10% were defined as having functional somatic symptoms and 70% had somatization associated with a mood or anxiety disorder. Digestive symptoms were reported in 30% of patients, headaches occurred in 25%, and unusual sensations in 20%. In patients of a younger age, there was no correlation between MUS and the patients' academic background, reason for visiting the medical center, diagnosis, symptoms, presence or absence of a referral form, continued treatment after the first visit to the medical center. However, a positive correlation did exist between MUS and the reason for visiting the medical center, referral form, and medical history after the first visit.

Conclusion: Many patients that present with MUS, regarding age, were referred to us from other hospitals and continued to receive medical services at the university hospitals. We concluded that patients who were difficult to diagnose or treat were referred the Department of Psychosomatic Medicine at Japanese university hospitals; therefore, these hospitals must prevent mistaken diagnoses by conducting effective psychological treatment and thorough medical examinations.

Keywords: Functional somatic symptoms (FSS); Medically unexplained symptoms (MUS); Somatization.
Glucocorticoid therapy causes contradictory changes of serum Wnt signaling-related molecules in systemic autoimmune diseases

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Background: The objective of this study was to investigate the clinical significance of the Wnt/β-catenin signaling pathway in glucocorticoid-induced osteoporosis.

Methods: A total of 91 patients with systemic autoimmune diseases who received initial glucocorticoid therapy with prednisolone (30-60 mg daily) were prospectively enrolled. We measured serum levels of N-terminal peptide of type I procollagen (P1NP), tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), sclerostin, Dickkopf-1 (Dkk-1), and Wnt3a before starting glucocorticoid therapy and every week for four weeks after its initiation. The effect of dexamethasone on expression of sclerostin and Dkk-1 by cultured normal human osteoblasts (NHOst) was evaluated by RT-PCR and ELISA.

Results: Serum levels of sclerostin and Dkk-1 increased significantly by one week and then decreased from the second week onward. Serum Wnt3a tended to decrease and serum P1NP showed a significant decrease. However, TRACP-5b was significantly elevated from the first week of treatment onward. In vitro study, dexamethasone increased Dkk-1 mRNA expression in cultured NHOst, but sclerostin mRNA was not detected. Dexamethasone also increased Dkk-1 protein production by osteoblasts, whereas sclerostin protein was not detected.

Conclusion: Bone formation might be impaired at least in the first week of the initiation of glucocorticoid therapy by increase of the serum Wnt signaling inhibitors, however, their reductions in the subsequent weeks were contradictory to the maintained suppression of the bone formation markers after glucocorticoid therapy for patients with systemic autoimmune diseases.

Keywords: glucocorticoid-induced osteoporosis, Wnt signaling
Interleukin-11 promotes epithelial-mesenchymal transition in anaplastic thyroid carcinoma cells through PI3K/Akt/GSK3β signaling pathway activation

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Background: Anaplastic thyroid carcinoma (ATC) is a lethal type of solid tumor, and metastasis is its major cause of treatment failure. In the preliminary clinical study, we demonstrated that interleukin (IL)-11 expression is positively correlated with distant metastasis in ATC. However, the mechanisms underlying remain largely unknown.

Methods: Real-time PCR and ELISA were performed for examining the IL-11 mRNA and protein expression in thyroid carcinoma cell lines or its supernatant, respectively. MTT assay was used to analyze the effect of IL-11 on the proliferation in ATC cells; Transwell and wound healing assays were employed to analyze the abilities of migration and invasion in ATC cells. Western blotting were used to detect the relative pathway protein.

Results: We found that cobalt chloride (a hypoxia mimetic) promoted IL-11 expression via HIF-1α activation. Furthermore, the resultant increase in IL-11 expression significantly induced epithelial-mesenchymal transition (EMT) in ATC cells, accompanied by Akt/GSK3β pathway activation and increased invasive and migratory abilities. Conversely, HIF-1α or IL-11 knockdown, or treating cells with a neutralizing antibody against IL-11, a PI3K inhibitor, or Akt inhibitor V, significantly suppressed the induction of EMT and counteracted the enhancements in invasive and migratory abilities.

Conclusion: These results indicate that hypoxia increases IL-11 secretion in ATC cells via HIF-1α induction and that IL-11 then induces EMT in these cells via the PI3K/Akt/GSK3β pathway, ultimately improving their invasive and migratory potential. This study elucidates the important role played by IL-11 in ATC metastasis and indicates it as a potential target for the treatment of cancer metastasis.

Keywords: Anaplastic thyroid carcinoma; Interleukin-11; Epithelial-mesenchymal transition; Metastasis
P-21

Epstein-Barr Virus Infection and Epstein-Barr Virus Nuclear Antigen 1 Variants in the Synovial Tissue of Rheumatoid Arthritis

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Background: Epstein-Barr virus (EBV) infection causes various malignant tumors, such as B cell lymphoma and epithelial cell cancer. Previous reports showed a certain variant of EBV nuclear antigen 1 (EBNA-1) may trigger development of the malignant cells. It was also reported that EBV infection may play a role of RA, however, underlying mechanisms remains unknown. Moreover, mutation of EBV in RA patients has not been examined. We then investigated EBNA-1 in the synovial tissue of RA.

Methods: One hundred twenty-eight RA and 98 osteoarthritis (OA) patients undergoing joint surgery at the Toho University Omori Medical Center were enrolled. Synovial tissues were collected during surgery under sterile condition. Informed consent was obtained from all the patients. DNA was extracted from the synovial tissues. The EBNA-1 gene was determined by nested PCR. The amplicons were detected by performing electrophoresis. Nucleotide sequence of the PCR product was determined. HLA DRB1 genotyping was also performed.

Results: EBV DNA was more frequently detected in the synovial tissue from RA (32.8%; 42 of 128) than OA (15.3%; 15 of 98) (p<0.01, chi-squared test). The sequence of EBNA-1 revealed Japanese prototype (V-Val subtype) in 35 of the 42 RA (83.3%) and 13 of the 15 OA (86.7%). Although four other subtypes were also detected in small number of patients, there were no significant differences between RA and OA. Frequency of HLA-DRB1*0405, *0410, *1001 (shared epitope: SE) was significantly higher in RA (55.5%) than OA (30.6%). Proportion of EBV-positive tended to be higher in SE-positive (39.4%; 28 of 71) than SE-negative (24.6%; 14 of 57), although it is not statistically significant.

Conclusion: EBV might be an environmental risk factor for development or chronic synovitis of RA. However, nucleotide mutations of EBNA-1 may not contribute it.

Keyword: Rheumatoid arthritis, Epstein-Barr virus, nucleotide sequence
P-22
IL-1, VDR and MMP-1 single nucleotide polymorphisms of chronic periodontitis in Wa population of Gengma

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Background: The aim of this study is to examine the possible association between single nucleotide polymorphisms of +3954C/T in IL-1B gene, -TaqIT/C in VDR gene and -16071G/2G in MMP-1 gene and chronic periodontitis susceptibility and severity of chronic periodontitis and severe chronic periodontitis susceptibility in Wa and Han population of Gengma.

Methods: Genomic DNA was obtained from the buccal swabs with the buccal mucosal epithelial cells of the subjects, IL-1B+3954 (C/T), VDR-TaqI(T/C) and MMP-1-1607(1G/2G) gene polymorphisms were genotyped using Sanger DNA sequencing method.

Results:
1 Analysis of polymorphisms showed no difference in distribution of the +3954 C/T variants in the IL-1B gene between Wa and Han population (P>0.05).
2 Analysis of polymorphisms showed no difference in distribution of the -TaqIT/C variants in the VDR gene between Wa and Han population (P>0.05).
3 Analysis of polymorphisms showed no differences in distribution of the -16071G/2G variants in the MMP-1 gene between Wa and Han population (P>0.05).

Conclusions:
1 Based on the results of this study, no significant association is found for the +3954 SNP in IL-1B gene with susceptibility of chronic periodontitis or severity of chronic periodontitis and severe chronic periodontitis susceptibility in these Wa and Han population.
2 Based on the results of this study, no significant association is found for the -TaqI SNP in VDR gene with susceptibility of chronic periodontitis or severity of chronic periodontitis and severe chronic periodontitis susceptibility in these Wa population.
3 Based on the results of this study, no significant association is found for the -1607 SNP in MMP-1 gene with susceptibility of chronic periodontitis or severity of chronic periodontitis and severe chronic periodontitis susceptibility in these Wa population.

Keywords: chronic periodontitis, IL-1, susceptibility
P-23

Multimodal Team approach to Failed Back Surgery Syndrome with Spinal Cord Stimulation

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Background: Failed back surgery syndrome (FBSS) is a condition in which improvement of symptoms is poor or worse after of spinal surgery. In the pain clinic, one of the innovative treatment is spinal cord stimulation therapy (Spinal Cord Stimulation: SCS), but evaluation methods have not been established.

Methods: From September 2015, team intervention was performed on five lumbar FBSS patients who underwent SCS. As an effect judgment of SCS, in addition to the degree of improvement of pain by our department, the gait function was evaluated in the Department of Rehabilitation.

Results: After SCS, 4 cases obtained pain relief, and also showed reduction of QOL-disability and improvement of intermittent claudication. Only one case described no change of pain score but showed highly improvement of gait performance. Numerical Rating Score (NRS) as a change (median ± SD) before surgery / three months after surgery: 7.8±2.9 / 2.8±1.9, 6 min walking test as walk function: 334±119 / 394±81 (m), Walking maximum speed: 77±19 / 85±23 (m/min). Roland-Morris Disability Questionnaire (RMDQ) and Oswestry Disability Index were showed a reduction in daily living disorders in all patients.

Conclusion: Comprehensive assessment focused not only on pain assessment but also on walking ability and daily living is effective for scientific demonstration of SCS.
P-24
Treated dentin matrix particles combined with dental follicle cell-sheet stimulated periodontal regeneration

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**Background:** The purpose of this study was to evaluate the effects of treated dentin matrix particles (TDMP) on human dental follicle cell sheets (DFCSs) and explore the possibility that TDMP combined with DFCSs may be useful for periodontal regeneration.

**Methods:** Fabrication of dentin matrix particles using grinding method and then undergo gradient demineralization, the physicochemical properties of TDMP were detected by laser particle analyzer, SEM, XRD, XPS, FI-IR, ELISA. Then, we assessed TDMP for the ability to support osteogenic differentiation of human BMMSCs in vitro and bone regeneration in rat critical-sized calvarial defects in vivo. Dental follicle cells were cultured in the cell sheet induction medium to create cell sheet. The effect of TDMP extracts on DFCSs were assessed by RT-PCR and WB. Finally, one wall intrabony defect model on Beagle dog were used to test the capacity to repair periodontium defects of combination of DFCSs with TDMP.

**Results:** The fundamental element of TDMP were hydroxyapatite, and it could constant release factors such as TGF-β, VEGF, BMP-2, PDGF-BB, and the extracted liquid of which could induce BMMSCs to proliferation and osteogenic differentiation. Meanwhile, it was showed that TDMP possess the abilities to repair bone defects. When DFCSs were induced by TDMP, it’s periodontal ligament-specific and osteogenic differentiation marker were increased or maintained. Importantly, eight weeks after the transplantation, periodontal regeneration was significantly observed with both newly formed cementum and well-oriented PDL fibers and alveolar bone more in TDMP+DFCSs groups.

**Conclusion:** These results indicate that DFCSs combined with TDMP scaffold serve as a promising tool for periodontal regeneration.

**Keywords:** periodontal regeneration, cell sheet, dentin matrix.
Maxillary Reconstruction Using Submental Artery Island Flap and Sagittal Mandibular Ramus/Coronoid Process Graft Pedicled with Temporalis Muscle

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Background: The purpose of this study is to demonstrate maxillary reconstruction using submental artery island flap and sagittal mandibular ramus/coronoid process graft pedicled with temporalis muscle through the modified lateral lip-submandibular approach.

Methods: From May 2013 to September 2016, eleven patients suffering from maxillary defects secondary to maxillary cancer ablation, who underwent maxillary reconstruction using submental artery island flap and sagittal mandibular ramus/coronoid process graft pedicled with temporalis muscle through the modified lateral lip-submandibular approach, were enrolled into this prospective study.

Results: All submental artery island flaps and sagittal mandibular ramus/coronoid process graft were successful with satisfactory function and esthetic outcomes. No functional impairment at the donor sites occurred in any cases.

Conclusion: The submental artery island flap combined with sagittal mandibular ramus/coronoid process graft is a feasible and acceptable technique for the maxillary reconstruction in older patients because it is safe, quick and simple to harvest, it offers a very acceptable esthetic and satisfactory outcome, with the advantage of low morbidity of the donor site.

Keywords: Submental artery island flap, Sagittal mandibular ramus, Coronoid process graft, Temporalis muscle
P-26
Alteration in plasma and striatal levels of D-serine after D-serine administration with or without nicergoline: an in vivo microdialysis study

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Background: D-Serine (D-Ser), a co-agonist of N-methyl-D-aspartate receptor (NMDAR), is effective for treating schizophrenia. In the present study, we investigated changes in plasma and striatal D-Ser levels in Sprague-Dawley (SD) rats after intraperitoneal D-Ser administration alone or together with nicergoline (Nic), a commercial cerebral ameliorating drug, using in vivo microdialysis (MD) to explore the pharmacological function of Nic.

Methods: Phosphate-buffered saline (PBS) or Nic (0, 1.0, or 3.0 mg/kg) followed by D-Ser (5.0, 10.0, 20.0, and 50.0 mg/kg for PBS or 20.0 mg/kg for Nic) was administered intraperitoneally to male SD rats, and the profiles of D-Ser levels in plasma and striatal MD samples were examined by high-performance liquid chromatography (HPLC) with fluorescence detection. The area under the curve (AUC) for the MD and plasma samples was also calculated and statistically compared among the groups.

Results: AUC values of D-Ser were increased in a D-Ser dose-dependent manner in plasma samples, while a proportional increase in the AUC values of striatal MD samples was only observed in D-Ser doses up to 20 mg/kg. The Nic co-administered group showed a significant increase in the AUCs of plasma D-Ser in a Nic dose-dependent manner, but the AUCs in striatal D-Ser were significantly decreased with increasing Nic doses, suggesting that Nic may prevent excess D-Ser from penetrating the central nervous system (CNS).

Conclusion: Nic may prevent an excessive distribution of exogenous D-Ser, such as that from a dietary origin, into the CNS to suppress an excitatory neurotransmission through NMDAR.

Keywords: D-Serine, Nicergoline, N-Methyl-D-aspartate receptor, Microdialysis, Schizophrenia.
P-27

Analyses of endogenous metabolites in serum from at-risk mental state subjects

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Background: It has recently been reported that the levels of some endogenous compounds were altered in serum of patients with schizophrenia as compared with those of healthy controls¹), and these compounds may be expected as biomarkers for schizophrenia. Schizophrenia reaches the onset as a prodrome via psychotic at-risk mental state (ARMS), however, there have been few reports on the serum concentrations of endogenous compounds in ARMS phase. In the present study, serum concentrations of endogenous compounds, L-tryptophan (L-Trp), L-kynurenine (L-KYN), 5-hydroxytryptamine (5-HT), D-, L-lactate, glucose, between ARMS and healthy subjects were compared.

Methods: Written informed consent was obtained from all subjects prior to participation, and the protocol was approved by both of the ethics committee in the Faculty of Pharmaceutical Sciences (No. 26-4) and the Faculty of Medicine (No.26012), Toho University. ARMS subjects (6 males and 8 females) and healthy volunteers (8 males and 17 females) were recruited with their informed consent. Glucose was measured using a commercial kit, glucose CII-test Wako. All compounds except glucose were determined by HPLC with fluorescence detection or LC-MS/MS.

Results: Although there were no significant differences of serum levels of 5-HT, L-KYN, glucose, and D-, L-lactate between ARMS subjects and healthy controls, the serum levels of L-Trp, a precursor of L-KYN and 5-HT, were significantly increased in the ARMS subjects (p=0.010).

Conclusion: The present results are consistent with previous report describing that the levels of serum L-Trp were significantly increased in patients with schizophrenia¹) and suggested some association between L-Trp and onset of schizophrenia. In addition, the correlation between levels of these compounds in serum and clinical scores of ARMS subjects are also presented.


Keywords: at-risk mental state, schizophrenia, tryptophan
Quick and easy preparation method for Kampo formula decoctions (part 4): IPCD method for rhubarb and aconite root

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**Background:** The immersing powdered crude drugs (IPCD) method has been developed as a quick, easy and high-yielding method for preparing decoctions. Given that the extraction process is different from the conventional decoction method, the safety of this method should be carefully examined. In the present study, the amounts of the diester alkaloids of aconite root, the tuberous root of Aconitum carmichaeli, and sennoside A of rhubarb, the rhizome of Rheum palmatum, were determined in the IPCD immersion. Both of these compounds have strong physiological activities and have been suggested to be decomposed during the decoction process.

**Methods:** The amounts of marker compounds in the IPCD immersion prepared from processed or unprocessed aconite root or rhubarb were measured and compared with those prepared using the conventional decoction method.

**Results:** The amount of sennoside A extracted with the IPCD method was 1.1-fold increased compared with that from the conventional method. No diester alkaloids were detected in the IPCD immersion prepared from processed aconite root, whereas for unprocessed aconite root, the amount of diester alkaloids extracted from the IPCD immersion was 8.8-fold higher than that from the conventional decoctions.

**Conclusion:** The IPCD method can be safely used on rhubarb or well-processed aconite root under the same conditions as other less-powerful crude drugs, whereas precautions are required for unprocessed aconite root.

**Keywords:** decoction, immersing powdered crude drug, processed aconite root, rhubarb
Application of AMSAT to Kampo examination: Prescription decision of Yokukansan and Kousosan

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**Background:** Prescription of Kampo medicines is decided by patterns from four examinations, including Inspection, Listening and smelling, Inquiry, and Palpation. Prescription decision of Kampo medicines is always difficult for whom not being a Kampo medical specialist. Thus, we paid attention to the application of "Auto Mastic System for Analysis Therapy" (AMSAT), which can visualize the function of the whole body, to Kampo examination. Herein, we reported the application of AMSAT in prescription decision of Yokukansan and Kousosan, which are representative Kampo medicines for the treatment of mental illness.

**Methods:** We choose patients who complained about anxiety and frustrating, and whose prescriptions were Yokukansan (also include Yokukansan-kachimpihange) (16 patients) and Kousosan (6 patients) in Kichijoji-Touhou Clinic. We checked their body function through "BASE" and "Colloidal-Shift". BASE is used for checking the function of whole body, and Colloidal-Shift is used for checking the hardness of cytoplasm.

**Results:** In all cases, Colloidal-Shift showed from yellow to red color at their head. The patients, who were prescribed Kousosan, showed stress level at 1-2. On the other hand, 8 out of 16 cases, who were prescribed Yokukansan, showed the highest stress level at 4, and BASE showed blue color at their head. This observation was not found in the cases of Kousosan. Among five re-visit patients, who were prescribed Yokukansan, they were found improvement of the stress level, and BASE was showed from blue to green or yellow at their head. This indicted the possibility of that therapeutic improvement by Yokukansan may be observed through AMSAT.

**Conclusion:** AMSAT maybe a useful tool for prescription decision of Yokukansan against mental illness.

**Keywords:** Kampo, AMSAT, Yokukansan, mental illness
Identification of caffeoylquinic acid derivatives as protein tyrosine phosphatase 1B inhibitors from *Artemisia princeps*

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Background: PTP1B ubiquitously expressed in classical insulin targeted tissues, and has received extensive attention as important target enzyme for the treatment of insulin resistance diseases, as the PTP1B plays critical roles in negatively regulating insulin- and leptin-signaling cascades. These have led to an intense interest in developing PTP1B inhibitors as potential therapies for diabetes. *Artemisia princeps* Pamp. (Asteraceae), is a widely distributed in Asian. It is used in traditional medicine for the treatment of inflammation, gastric ulcer, and many circulatory disorders. The aim of the present study is discovery of novel natural PTP1B inhibitors from *A. princeps*.

Methods: Aerial parts of *A. princeps* was extracted with MeOH and then separated with column chromatography and semi-preparative HPLC, which led to the isolation of ten caffeoylquinic acid derivative. The chemical structures of compounds were determined by NMR and MS spectra. The potentials of compounds as PTP1B inhibitors were evaluated by enzyme kinetic, molecular docking simulation, inhibitory selectivity against other PTPs (e.g., SHP-1, SHP-2, VHR and TC-PTP).

Results: All isolated compounds exhibited above 60% inhibition of PTP1B at 100 μM. These compounds inhibited PTP1B in a concentration dependent manner, and their IC$_{50}$ values were determined by regression analyses. Among them, chlorogenic acid (3) was the most potent (IC$_{50}$ 11.13 μM). Further investigation demonstrated that 3 is a noncompetitive inhibitor by kinetic analysis, and molecular docking simulation. 3 also showed inhibitory selectivity between PTP1B and omologous PTPs (SHP-1, SHP-2, and VHR).

Conclusions: Chlorogenic acid from *A. princeps* is a natural PTP1B inhibitor and might be potentially valuable for further investigation of anti-diabetic effect.

Keywords: Caffeoylquinic acid, Protein tyrosine phosphatase 1B, *Artemisia princeps*
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*Simultaneous Quantification of Surfactants in Environmental Samples by LC-MS*

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**Background:** Recently, surfactants are more frequently synthesized in larger quantities. Even after water treatment, a part of these compounds ends up in surface waters. Although there is no evidence for accumulation, the determination of surfactants is important for environmental analysis. In this study, we developed a simultaneous determination method for cationic surfactants (CS), anionic surfactants (AS) and nonionic surfactants (NS) in river water, sediment and shellfish using liquid chromatography mass spectrometry (LC-MS).

**Methods:** Standard solutions were prepared ranging between 250 - 2000 ppb for AS, 0.9 - 18 ppb for CS and 200 - 800 ppb for NS. For analysis, LC-MS with electrospray ionization in positive and negative modes was used. The column used was a Shodex MSPak GF-310. A gradient elution method was used with acetonitrile. In selected-ion monitoring (SIM), [M]+, [M]− and [M+Na]+ were used for detection of AS, CS and NS, respectively. Solid phase extraction (SPE) was used as pre-treatment to separate surfactants from sample matrix. For validation, among other, recovery after SPE treatment using standards was determined. Environmental samples were collected from Kisarazu-shi, Chiba-ken.

**Results and Conclusion:** 3 CS, 4 AS and 14 NS compounds are simultaneously determinable with the developed method. Validation showed poor range for 1 NS, while recovery was positive for the rest. CS and AS ions, [M]+ and [M], were detected by ESI+ and ESI− at retention times between 9 and 13 min and between 26 and 31 min, respectively. The sodium adduct ions [M+Na]+ of NS were detected by ESI+ at the retention times between 8.5 and 29.5 min. Surfactants were successfully extracted with the SPE method and showed possible accumulation of CS in sediment and Asari shellfish.

**Keywords:** surfactants, quantification, environmental
P-32
Concentration of PAHs in the Excrement of Annelids

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Background: Polycyclic aromatic hydrocarbons (PAHs) occurred in any combustion processes and derived from fossil fuels diffuse in the environment. Some of PAHs are mutagens, carcinogens, or endocrine disruptors and hence it is important to grasp the kind and amount of hazardous PAHs and to recognize their movement in the environment.

In a previous study, we measured PAH concentrations in sediments and in benthos and their excrements, which were collected from the Yoro tidal flat in Japan. In the course of the study, we found interesting phenomena, bioaccumulation and biodegradation of PAHs in the excrement of an annelid, *Marphysa sanguinea*.

In the present study, to clarify the bioaccumulation feature of PAHs in the excrement of *M. sanguinea*, we analyzed the PAH concentrations in sediment, excrements after excreted by annelid, and excrements in annelid’s body.

Methods: Samples; sediments, annelids and excrements; were collected in the Yoro tidal flat (Ichihara, Chiba, Japan). These samples were pretreated by acetone extraction, alkaline decomposition, and hexane extraction, and purified by silica gel column chromatography. Concentration of PAHs in samples was measured by gas chromatograph - mass spectrometer (GC-MS). Phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene, and perylene were selected as target compounds. Deuterium-labeled PAHs were used as surrogate standards for determining the recovery rates of PAHs in the sample preparation procedure. *p*-Terphenyl- *d*14 was used as an internal standard.

Results and Conclusion: PAH concentrations of samples, sediments and excrements of annelids, significantly differed according to the sampling date and location. The excrement of annelid contained PAHs at concentrations approximately 8 to 200 times that found in the sediments. These results indicate that the accumulation of PAHs is usually occurred in the excrements of *Marphysa sanguinea*.

Keywords: polycyclic aromatic hydrocarbons, bioaccumulation, GC-MS
Biodegradation of PAHs by Microorganisms Isolated from the Excrement of *Marphysa sanguinea*

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**Background:** Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants generated during the incomplete combustion of organic materials. Since PAHs are potential mutagens, carcinogens, or endocrine disruptors, it is important that they are degraded effectively. In our previous studies, we discovered an interesting phenomenon in which PAHs showed rapid degradation in the excrement of *Marphysa sanguinea*.

To clarify the mechanism underlying the rapid degradation of PAHs in the excrement of *M. sanguinea*, pyrene (Pyr) -degrading microorganisms were isolated and examined their abilities to degrade PAHs in vitro.

**Methods:** Excrements of *M. sanguinea* were collected in the Yoro tidal flat (Ichihara, Chiba, Japan), and Pyr-degrading microorganisms were isolated from the excrement using a selected medium containing Pyr as the sole carbon source. Isolates cultured on the agar plates containing 50 mg L\(^{-1}\) Pyr and incubated at 28°C for 7 days. Then, the colonies of isolates were suspended into a liquid medium without pyrene. Samples were extracted from the medium with 1 mL of hexane and acetone after 0, 3, 8, 10 days, and the concentrations of Pyr in samples were monitored by gas chromatograph - mass spectrometer (GC-MS). GC-MS analyses were performed on a Shimadzu GC-2010 equipped with a capillary column (Rtx-5MS, 30 m length, 0.25 mm diameter).

**Results and Conclusions:** Pyr levels were gradually decreased in the media in which these microorganisms were cultured. Approximately 20–70% of the pyrene was removed after 9 days of incubation and 1-hydroxypyrene, a pyrene metabolite, was detected in the media. The pyrene degradation ratio of individual microorganisms in the media was lower than that in the excrement; thus, these microorganisms likely comprise a part of a pyrene-degrading consortium in PAH-polluted environments.

**Keywords:** polycyclic aromatic hydrocarbons, biodegradation, *Marphysa sanguinea*
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Taste masked technique of *Andrographis paniculata* extract for lozenges formulation

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**Background:** *Andrographis paniculata* has been used for treatment of uninfected sore throat and diarrhea. Due to its bitterness and high taking amount easily made the patients lost their compliance. As part of our interest in development of *A. paniculata* lozenges formulation, this study aimed to taste masked of *A. paniculata* extract for further formulation and the released parameter was also investigated.

**Methods:** *Taste masking:* *A. paniculata* ethanolic extract was taste masked with 1) solid dispersion with chitosan and 2) calcium alginate beads hydrogel forming. Drug:polymer ratio were varied from 3:1 to 1:3. *Evaluation of bitterness:* The taste masked extracts were tasted and compare the bitterness to standard quinine hydrochloride solutions. *Release profile:* The andrographolide releasing profile of the hydrogel taste masked extract was evaluated using dissolution apparatus for the first 2 hr at pH 2.0 and further 6 hr at pH 6.8.

**Results:** It was found that only calcium alginate bead hydrogel forming was able to masking the bitterness of the extract. The most appropriate drug:polymer ratio was found at 2:1. The tester did not feel any bitterness from the hydrogel. This might be due to the hydrogel cover all the extract into sphere shape protect the taste bud from the bitter extract. The release profile was shown in the figure. It was found that the pellet bead itself exhibited delayed release profile. This may be due to the calcium alginate bead has broken up in higher pH.

**Conclusion:** Calcium alginate hydrogel forming was the most appropriate taste masking technique for *A. paniculata* extract. Its release profile was delayed release pattern and suitable for further lonzenges formulation.

**Keywords:** taste masked, calcium alginate beads, andrographolide
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Needs Survey of Faculty Members at Educational and Research Institutions on Child-Raising and Elderly Care

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Background: Toho University have put effort into support activities on child-raising and elderly care throughout female physician supports and promotion activities of gender equality. Our university adopted many kinds of support systems such as dispatch program of research support staff, and sick child care services prior to other universities in Japan. However, there are still many issues to keep a good balance among work and child-raising and elderly care. In Japan, female researchers account for only a small portion in the overall faculty members. Our university was adopted “Program to supporting research activities of female researchers” with two institutions in 2015, and this opportunity enables us to facilitate female researches supports. Three institutions conducted a needs survey on child-raising and elderly care, and the present poster shows results of Toho University. The purpose of the survey was to clarify things necessary for good balance between work and home life.

Methods: The survey was conducted in 2017, and 833 faculty members participated. They were asked to answer self-completed anonymous questionnaires (41 questions) that included demographic data and needs for child-raising and elderly-care using a three or four-point scale.

Results: The response rate was 26.9% (224 faculty members), and 37.5% (84 faculty members) were female. The highest response rate was 42.4 % among the age group of 41-50 years old. One hundred and seventy-five (78.1%) were married, 154 (68.7%) have more than one child, and 56 (25%) have a family with needed long-term care. Female subjects feel that there are “needs” regarding child-raising and elderly-care rather than male subjects (independent t-test). Also, the results suggest understandings of family members and workplaces are placed great significance on child-raising as well as child care support systems such as a nursery school and a parental leave. Moreover, the results suggest understandings and actual support of family members and understandings of workplaces are placed great significance on elderly care. Finally, results of independent t-tests showed that more female subjects feel very difficult balancing their family and job especially in child-raising of school children and elderly care.

Keywords: women researchers’ support plan, Needs Survey, Child-raising, Elderly-care
Toho University started as the Imperial Women’s Medical College in 1926. It has since laid the foundation for Japanese women to receive higher education in medicine and biological science. The University has produced many talented women researchers including Dr. Katsuko Saruhashi who established the Saruhashi Award to give to women who made their distinguished research results. The University has been still developing the educational system for highly-skilled medical professionals such as doctors, pharmacists, nurses, and others engaged in the natural science field.

According to the Basic Law on Science and Technology promulgated in 1995, Japan has settled on a concrete 5-year support plan for women researchers. In 2008, the University set up the support office for women, and established the program to help “junior doctors on training” who tend to leave their workplaces, influenced by the idea of “All or None.”

In 2009, under the initiative of “Promoting Role Models to Support Female Researchers” of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the University started the “day care center for children with infections,” a “discount coupon for babysitting services” and a temporary staffing service sending an “assistant” to women researchers handling both a career and raising children.

In 2015, the University collaborated with Chiba University and National Institutes for Quantum and Radiological Science and Technology to form a tripartite project for supporting female researchers. Adopted in “the Program to Support the Research Activities of Female Researchers of MEXT,” this is now the core of a Consortium. In April, 2017, we renamed into “the Center for Diversity and Inclusion” to make our efforts known more widely.
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The Contribution of International Students Education to the Internationalization of Medical Education ——Taking Kunming Medical University as Example

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Background: The education for international students grows rapidly at Kunming Medical University in the last 5 years and promotes the process of internationalization of the university. Taking KMU as example, this thesis is to analyze how international students education contribute to the internationalization of medical education, so as to summarize successful experience, introspect the difficulties and problems, and to put forward suggestions.

Methods: The thesis analyzes the education of international students is good for the internationalization of faculties, teaching, scientific research, and university culture, promoting the internationalization of the whole university; and introspects the difficulties and challenges like the quality of enrolled students, the guarantee of policies and regulations, and the quality supervision of education etc. It also put forward the suggestions to upgrade the international students education as to expand international communication for optimization of student resource, foster management and enhance education quality, and developing a group of high-quality teachers and management faculties.

Results and Conclusion: To take the opportunities and advantages of international students education, the medical education could enhance its international competitiveness and contribute to the internationalization of medical education.

Keywords: international education, internationalization