PROCEEDINGS OF

THE INAUGURAL AAPS-NUS STUDENT CHAPTER SYMPOSIUM

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Message from the Chair

Welcome to the Inaugural AAPS-NUS Student Chapter Symposium.

The American Association of Pharmaceutical Scientists (AAPS) is a professional, scientific society of more than 12,000 members employed in academia, industry, government and other research institutes worldwide. The National University of Singapore (NUS) is one of the finest universities in the Asia-Pacific region. The AAPS-NUS Student Chapter is a non-profit student organization, which facilitates local student participation in the activities of AAPS and increases student awareness of career opportunities and latest advances and discoveries in the pharmaceutical sciences.

The program for this meeting is very interesting, featuring about 15 posters and 10 podium presentations by chapter members and invited speakers from academia and industry. It will provide a good opportunity for students, principal investigators and employers to mingle.

Best Presenter Awards will be given to the outstanding presenters at both poster and podium sessions. By doing so, we hope to spur everyone's interest and pursuit for notable accomplishments in pharmaceutical research.

I would like to thank everyone who have made this meeting a truly memorable experience for all participants.

Regards,
Lifeng Kang
Chairperson

AAPS-NUS Student Chapter Executive Committee 2005/06

Chair	Lifeng Kang
Chair-elect	Ai Ling Ching
Vice Chair	Josephine Soh
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Faculty Advisor	Associate Professor Sui Yung Chan

Inaugural AAPS-NUS Student Chapter Symposium Program

1130-1255 Lunch and poster viewing

1255-1300 Introduction by A/P Chan Sui Yung , Student Chapter Faculty Advisor

1300-1325 Ms Tan Lai Lee, Clinical Director Quintiles and NUS Pharmacy Alumni *Clinical Research as a Career Option - Challenges and Opportunities for Pharmacists in Clinical Research*

1325-1350 Dr Malini Olivio, Principal Scientist National Cancer Centre and NUS Pharmacy Adjunct Assoc Professor. *Cancer detection and therapy using novel photosensitizersa and bioimaging*

1350-1415 A/P Lawrence Ng, NUS Pharmacy newest faculty. *His research interest and move to Singapore from USA*

1415-1440 Mr Gwee Pai Chung, NUS Pharmacy Alumni, PhD candidate in Biochemistry. *The Hitchhiker's Guide to Graduate School*

1440-1530 Chapter member presentations

1530-1535 Closing speech by Mr Kang Lifeng, Student Chapter Chairperson

1535- 1600 Tea

Poster Presentations

1) Transdermal drug delivery and Surface Tension

Fathi-Azarbayjani A

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Delivery of the drug via the skin has many advantages over conventional methods. However due to the barrier presented in the skin, many strategies have been suggested in order to overcome this barrier. A popular approach is the use of penetration enhancers, such as surfactants. The increase in flux is due to the ability of the surfactant molecule to adsorb onto the surface of the skin and increase solubility and eventually solubilizing the extract lipid components. In other words, surfactants work by lowering the surface tension of the formulation and miscuing it with skin which helps the formulation to spread easily on the skin and penetrates rapidly onto the stratum corneum. One important research area is the calculation of surface tension. Experimental determination of surface tension is not possible for all mixture composition at various temperatures. However different techniques are used to study interactions between liquid surfaces, but many problems are so complex that it is impossible to study them analytically. For such problems, the use of mathematical representations may be an interesting complement to experimental investigations. We have proposed a model to calculate the surface tension of pharmaceutical solvents mixtures at various temperatures.

Methods: A simple computational method for calculation surface tension of solvent mixtures at various temperatures based on Redlich-Kister extension was proposed. The model (Jouyban-Acree model) was applied to the experimental surface tension of solvent mixtures and showed accurate results.

Results and discusssion: The accuracy of the proposed model has been compared with those of previously published models and results showed that the proposed model were superior and capable of providing more accurate results.

Conclusion: Nearly all technical applications within pharmaceutical require surface chemical considerations. In this sense surface chemistry is truly a cross-disciplinary research area. By applying special surface treatments to transdermal formulations, the distribution and dissolving behavior of the drug can largely be influenced. This enables the development of more safe and effective time-released pharmaceuticals. The proper choice of surfactant system is not possible without good understanding of adsorption phenomena and interactions between molecules adsorbed at the interface. Therefore, a good theoretical model of interfacial tension in mixed systems is required.

2) Combined St John's Wort Alleviates Dose-limiting Toxicities of Irinotecan in Rats

Hu ZP, Yang XX, Ho PC, Chan SY, and Zhou SF

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Irinotecan (CPT-11) is a DNA topoisomerase I inhibitor for advanced colorectal cancer therapy, whereas St John's wort (*Hypericum perforatum*, SJW) is a commonly used herbal antidepressant. This study aimed to investigate whether co-administered SJW modulated the toxicities of CPT-11 and the underlying mechanisms.

Methods: Healthy male rats were treated with CPT-11 alone for 4 consecutive days, or in combination with SJW for 8 consecutive days starting one day before the first CPT-11 injection. Blood cell counts, body weight, the incidence of diarrhea as well as macroscopic and microscopic intestinal damages were monitored throughout the study.

Results and Discussion: Rats treated with CPT-11 alone experienced significant decrease in body weight and numbers of neutrophils and lymphocytes with severe acute and delayed-onset diarrhea. In addition, marked pathological damages including swelling, hemorrhage and microscopic findings were observed in the intestine (in particular cecum) in rats treated with CPT-11. Coadministration of SJW with CPT-11 resulted in lesser body weight loss, reduction of numbers of neutrophils and lymphocytes and diarrhea compared to rats receiving CPT-11 alone. In addition, the intestinal damages induced by CPT-11 were significantly reduced in rats receiving the combination therapy.

Conclusion: Coadministered SJW significantly ameliorated the toxicities induced by CPT-11. The protective effect of SJW may be due to both pharmacokinetic and pharmacodynamic mechanisms and further studies are ongoing at our laboratory.

3) Rapid Sensor System for Ginseng Identification

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Objective: Quality of pharmaceutical products like ginseng is important for ensuring consumer safety and efficacy. Many ginseng products sold today are in various formulations such as powder, capsules, tablets, and tea. This renders it less identifiable by morphological means. Furthermore, as ginseng is expensive, adulteration with other cheaper products occurs. Hence quality assurance of ginseng is needed.

Method: The sensitivity and high throughput of FTIR spectrometers can be combined with high optical efficiency fiber-optic materials to develop an optimized biosensor tool for analysis. PCA was carried out on the second derivative IR spectra of American and Asian ginseng samples to differentiate them, and to distinguish them from sawdust - a morphologically similar adulterant.

Results and Discussion: The fingerprint region (2000-600cm-1) can be used to distinguish the ginsengs from sawdust, as well as to differentiate between the ginsengs. All three samples were able to form distinct clusters in the 2-D plots. However, the ginseng samples formed a closer cluster with each other than with sawdust. Thus the differences in chemical composition between sawdust and ginseng can be easily identified via their IR spectra. The greater variation in the Asian ginseng spectra can be due to different growing environments and cultivation conditions which may affect the amount of active constituents.

Conclusion: The main advantages of this technique are the system's rapid detection speed, minimum sample preparation and non-destructive nature of the interrogated sample. Despite its potential, there are currently no or limited commercial exploitation of fiber optic-based sensors to perform ginseng quality analysis. Hence, the opportunity for biosensors to be used in ginseng analysis is definitely appealing.

4) Determination of Dencichine, a Haemostatic Agent, in *Panax* species using Liquid Chromatography-Tandem Mass Spectrometry

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Objective: Dencichine (β-N-oxalyl-L-α,β-diaminopropionic acid) is an active haemostatic agent found to be present in raw and steamed *Panax notoginseng*, as well as other *Panax* species such as *Panax quinquefolium* and *Panax ginseng*. It is also a known neurotoxic agent found in *Lathyrus sativus* (grass pea seeds). To date, no Liquid Chromatography Mass Spectrometry (LC-MS) methods have been reported for the analysis of this small and highly polar compound. Therefore, the objective of this study is to develop a suitable LC-MS/MS method for the analysis of dencichine in *Panax* species.

Method: Using hydrophilic interaction chromatography coupled with positive electrospray ionisation tandem mass spectrometry (HILIC/ESI-MS/MS), underivatised dencichine was selectively detected. The content of dencichine in raw and steamed *Panax notoginseng* roots, 11 pairs of raw and steamed *P. notoginseng* herbal products, *Panax ginseng* roots, and *Panax quinquefolium* roots were analysed and compared.

Results and Discussion: Optimal sensitivity of 0.3 ppm (detection limit) and 1.5 ppm (quantification limit) was achieved. The method was selective and rapid, with the peak eluting at about 1 min. Compared to conventional methods, this technique eliminates the need for sample derivatisation. Steamed *P. notoginseng* samples were found to have less dencichine than the raw *P. notoginseng* samples. It has been postulated that dencichine is hydrolysed at high temperatures during steaming process. On the other hand, *P. ginseng* and *P. quinquefolium* were also found to contain less dencichine than the raw *P. notoginseng* species.

Conclusion: A novel LC/MS/MS method for the determination of dencichine has been developed and validated. With the presence of haemostatic dencichine in *Panax* species, there may be potential drug-herb interactions. This rapid analytical method will thus be important for the safety and quality control of dencichine content in complex medicinal plants and their products.

Design of cyclic peptides from CD2 epitopes for immunomodulation

Li C and Jois SDS
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Purpose: T cell surface protein CD2 plays important roles of adhesion and signal transduction in immune response. Blockage of CD2-CD58 interaction is expected to lead to therapeutic agents for autoimmune diseases or transplantation. The aim of this study is to design peptides from CD2 binding epitopes to modulate CD2-CD58 interaction.

Methods: 12 amino acid cyclic peptides have been designed from rat CD2 and human CD2 epitopes and subjected to truncation. Biological activities have been evaluated by cell adhesion assays (heterotypic adhesion assay and E-rosetting assay) and the secondary structures have been studied by circular dichroism (CD) spectroscopy.

Results: Our results indicated the minimum inhibitory segments (MIS) in the 12 amino acid peptides by cell adhesion assays. CD spectra suggested that some cyclic peptides adopt β turn structure in solution.

Conclusion: Cyclic peptides designed from CD2 epitopes could inhibit CD2 mediated cell adhesion by mimicking native β turn structure.

6) Synthesis of Bicyclic Diamino-triazaspiroalkenes as Potential Bovine DHFR Inhibitors

Ma X and Chui WK

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Dihydrofolate reductase (DHFR) is an important target for the drug development against cancer and a variety of infectious diseases caused by bacteria, protozoa and fungi. The 4,6-diamino-2,2-dimethyl-1,2-dihydro-1,3,5-triazines are known to be inhibitors of the DHFR for many years. It was found that bicyclic diamino-triazaspiroalkenes, having the dihydro-1,3,5-triazine nucleus, have not been extensively investigated for their antifolate activities. The objective of this study was to investigate the change of antifolate activity with respect to the modification at N_1 and C_2 position of the dihydro-1,3,5-triazine nucleus.

Methods: Diamino-N-phenyl-triazaspiroalkenes were synthesized by a three-component synthesis using aniline, cyanoguanidine and cycloketones. Diamino-N-benzyl-triazaspiroalkenes were cyclized using cycloketones and benzylbiguanides. Diamino-N-benzyloxy-triazaspiroalkenes were synthesized using the three-component synthesis by O-aminobenzyl alcohol hydroxchloride, cyanoguanidine and cycloketones; Diamino-N-phenoxyalkyloxy-triazaspiroalkenes were synthesized by alkylation of N-hydroxytriazines. The antifolate activity was determined using a spectrophotometric-enzyme assay that involved the use of commercially available bovine DHFR.

Results and Discussion: All 12 bicyclic diamino-triazaspiroalkenes were synthesized with reasonably good yields (21.2-75.9%) and successfully characterized. Compounds **1**-**4**, having phenyl or benzyl group on N position, were devoid of inhibitory activity. However, inserting an oxygen between the benzyl group and N position of the triazaspiroalkenic nucleus was able to improve the activity. Elongation of side chain of triazaspiroalkenes with 3-(phenyloxy) propyloxy substituent demonstrated very potent inhibitory activity against bovine DHFR. Spiro ring size of the triazaalkenes was also found to affect the activity, with larger ring size being less active: cyclopenyl > cyclohexyl > cycloheptyl. Compound **8** showed inhibitory activity with IC_{50} value of 0.002 μM, comparable to the positive control, MTX. Further elongation of side chain with 4-phenyoxybutyloxy substituent weakened the inhibitory activity. In summary, the flexible oxygen bridge found in compounds **8**-**10** could have allowed the molecules to bind at the active site of the enzyme comfortably and thus escape the steric hindrance caused by properties of the enzyme structure.

Conclusion: 7,9-Diamino-phenoxypropyloxy-6,8,10-triazaspiro[4.5]deca-6,8-diene (**8**) showed potent antifolate activity against bovine DHFR with IC_{50} value of 0.002 μM, comparable to methotrexate (MTX). Therefore the new antifolate **8** may have value in being used as an anticancer agent.

7) The Influence of Comminution Method on Physical Properties of Plant Matrix and its Impact on Continuous Countercurrent Extraction

Ooi SM, Chan LW and Heng PWS

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Botanical materials are often required to be comminuted to smaller particle size to facilitate mass transfer during extraction. Cut milling and impact milling are two common comminution methods that employ different fracture mechanisms and produce particles of different physical properties. It is also possible that the particles are "bruised" to different extents with different fracture mechanisms. Bruising may disrupt the integrity of plant matrix, thereby facilitating penetration of solvent into material for extraction. This study was undertaken to investigate the influence of cut milling and impact milling on the efficiency of continuous countercurrent extraction of a model botanical.

Method: Sliced licorice roots were comminuted at different milling speed using a Fitz mill. The sizing of comminuted samples was carried out using sieving method. Samples with comparable MMD (Mass Median Diameter) value obtained by cut milling and impact milling separately were then extracted using a pilot scale horizontal screw continuous countercurrent extractor under different extraction conditions, comprising high and low levels of process time, temperature and solvent flow rate.

Results and discussion: Markedly lower MMD values were obtained when the milling speed increased while the effect on Span was variable for both milling methods. Samples with smaller MMD value were obtained for impact milling at the same speed as cut milling. The yield of the comminuted sample obtained by impact milling was higher compared to cut milling when the extraction process time was shorter. This can be attributed to the bruising effect of impact milling, which facilitated the penetration of solvent into the plant matrix.

Conclusion: Extraction efficiency of continuous countercurrent extraction is affected by the milling method employed. Impact milling produces particle of smaller size with narrower size distribution and create a bruising effect on the particles. This bruising effect exerts greater influence when the process time is short.

8) Limonene Organogel as a Vehicle in the Transdermal Delivery of Haloperidol

Lim PFC [a], Liu XY [b], Kang L [a], Ho PCL [a], Chan SY [a]

Departments of Pharmacy [a] and Physics [b], National University of Singapore

Objective: A penetration enhancer dispersed in a viscoelastic organogel could serve as a long-acting formulation that delivers haloperidol (HP), an anti-psychotic drug, at a sustained percutaneous rate. Gelator GP1 in propylene glycol (PG) constitutes the microscopic framework of this transdermal vehicle. A branched network of interlocking fibres restricts HP diffusion on the vehicle side. Varying GP1 content modulates the gel stiffness which presents different degrees of resistance to drug transport. To overcome the skin barrier, limonene, a penetration enhancer, is incorporated into the gel.

Methods: In human skin permeation conducted at 37 ^{o}C, several gel formulations ([GP1] = 0 to 10 %w/v) was loaded into the donor compartments of Franz diffusion cells. HP in the receptor solutions was quantitated with a validated high performance liquid chromatograph (HPLC) assay. Advanced rheometric expansion system (ARES) characterized gel rheology – elastic modulus G' and viscous modulus G'' – at 32 ^{o}C and 1 Hz.

Results and Conclusion: As GP1 concentration increases, both partition parameter KL and permeability KD/L decrease, while lag time t_L remains unchanged. The implications are that GP1 itself does not hinder the activity of limonene, and the gel mesh-like structure poses an additional transport resistance through an effective immobilization of the solvent PG. To circumvent inconsistencies associated with different skin models and skin obtained from different anatomical sites, a gel resistance-rheology model was proposed. Rheology of the gel matrix decelerated HP delivery according to several empirically-derived relationships. These correlations supported the viability of the use of organogel in controlling drug release rate. The organogel, in addition to being a drug reservoir in a transdermal patch for instance, could act as a rate-controlling matrix.

9) Human Multidrug Resistance Associated Protein 4 Confers Resistance to Camptothecins

Tian Q[1], Zhang J[1], Tan MCT[2], Chan E[1], Duan W[2], Chan SY[1], Zhou SF[1]

[1]Department of Pharmacy, Faculty of Science, National University of Singapore;

[2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

Purpose: The multidrug resistance associated protein 4 (MRP4) is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family. Irinotecan (CPT-11), a camptothecin (CPT) derivative, is a potent DNA topoisomerase I inhibitor with a broad spectrum of antitumor activity. Both CPT-11 and SN-38 (the active metabolite of CPT-11) have been identified as substrates for P-glycoprotein (PgP) and MRP1/2. In this study, we explored the resistance profiles and intracellular accumulation of a panel of CPTs in HepG2 cells with stably over-expressed human MRP4.

Methods: HepG2 cells were transfected with an empty vehicle plasmid (V/HepG2) or human MRP4 (MRP4/HepG2). The resistance profiles of test drugs in exponentially growing V/HepG2 and MRP4/HepG2 cells were examined using the MTT assay with 4 hr or 48 hr exposure time of the test drug in the absence or presence of various MRP4 inhibitors. The accumulation of CPT-11, SN-38 by cells was determined by validated high performance liquid chromatography (HPLC) methods.

Results: Based on the resistance folds from the MTT assay with 48 hr exposure time of the test drug, MRP4 conferred resistance to CPTs. Overall, overexpression of MRP4 increased the IC_{50} values 1.78−14.21 folds for various CPTs in lactone or carboxylate form. The resistance of MRP4 to various CPTs tested was significantly reversed in the presence of DL-buthionine-(S,R)-sulphoximine (BSO), MK571, celecoxib, or diclofenac. In addition, the accumulation of CPT-11 and SN-38 over 120 min in MRP4/HepG2 cells was significantly reduced compared to V/HepG2 cells, while the addition of celecoxib, MK-571 or BSO significantly increased their accumulation in MRP4/HepG2 cells. MRP4 also conferred resistance to. However, MRP4 did not increase resistance to paclitaxel, carboplatin, etoposide (VP-16), 5-fluorouracil, and cyclosporine.

Conclusions: Human MRP4 rendered significant resistance to cyclophosphamide, CPT, CPT-11, SN-38, rubitecan and 10-hydroxy-CPT. CPT-11 and SN-38 are substrates for MRP4. Further studies are needed to explore the role of MRP4 in resistance, toxicity and pharmacokinetics of CPTs and cyclophosphamide.

10) Chlorin e6-polyvinylpyrolidone as a fluorescent marker for photodynamic diagnosis of human bladder cancer using the chick chorioallantoic membrane model

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Introduction: The study of fluorescence diagnosis as a modern cancer diagnostic modality is rapidly gaining importance in the field of urology. It is based on the detection of distinctive light emission of tissues sensitized by fluorescent dyes, commonly referred to as photosensitizers, after irradiation with a specific light source. Therefore the use of specific fluorescent dyes for bladder cancer is constantly being sought after.

Objective: The aim of this study is to investigate the use of a new formulation called Fotolon®, a mixture of chlorin e6 and polyvinylpyrrolidones (Ce6-PVP) for the detection of human bladder cancer using the chick chorioallantoic membrane (CAM) model.

Methods: Uptake kinetics studies were quantitatively determined for both systemic and topical administrations of Ce6-PVP to the normal CAM as well as the MGH human bladder tumor xenografted CAM using fluorescence imaging technique.

Results: Rapid elimination of Ce6-PVP was displayed 1 h following topical application compared to systemic administration in the normal CAM system. Ce6-PVP localized selectively in the xenografted bladder tumor as well as in the vasculature of the CAM. Neither dark toxicity nor irritancy was observed with CAM at the dose of 2 mg/kg used indicating that the formulation tested was acceptable for instillation in the bladder mucosa.

Conclusion: Ce6-PVP appeared to have the potential as a screening agent for bladder cancer.

11) Multidrug Resistance Associated Protein 4 Confers Resistance to Topotecan

Wong SAS, Tian Q, and Zhou SF

Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore

Objective: Topotecan is a water soluble CPT derivative showing modest activity in previously treated patients with ovarian and small cell lung cancer. In *Mrp4* knockout mice, topotecan brain concentration was significant increased compared to the wide type, indicating that topotecan is a potential substrate for mouse Mrp4. In this study, an attempt was made to examine whether overexpression of human MRP4 modulated the cytotoxicity of topotecan in the human liver carcinoma cells, HepG2 cells.

Methods: HepG2 cells were transfected with *MRP4* cDNA or empty vector and cultured in the DMEM medium containing 100 units/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum, in the presence of 0.25 μg/ml blasticidin. All cells were grown at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The cytotoxicity of topotecan to the HepG2 cells was examined using MTT assay with drug exposure for 4 hr or 48 hr. The effects of BSO (an glutathione synthesis inhibitor) on the cytotoxicity of topotecan in vector- and MRP4-transfected HepG2 cells were also investigated.

Results and Discussion: Overexpression of MRP4 conferred 12.03- and 6.87-fold resistance to topotecan in the 4-hr and 48-hr exposure assay, respectively. Compared to V/HepG2 cells, the presence of BSO in MRP4/HepG2 cells significantly decreased the cytotoxicity of topotecan to 2.21- and 2.19-folds in 4- and 48-hr exposure assay, respectively. The present study revealed that overexpression of MRP4 increased resistance of tumor cells to topotecan. BSO inhibited MRP4-mediated cellular resistance to topotecan.

Conclusion: Topotecan is highly likely a substrate for human MRP4. Topotecan has become one of the most widely used agents for salvage therapy in patients with ovarian carcinoma and MRP4 has been detected in ovarian tumor tissue, it would be likely that ovarian cancer cells render resistance to topotecan through MRP4 in humans. Further studies are needed to identify the relationship between MRP4 level and topotecan response.

12) Thalidomide Alters the Pharmacokinetic of CPT-11 and A Mechanistic Study

Yang XX, Hu ZP, Chan SY, and Zhou SF

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Thalidomide, a tumor necrosis factor-α inhibitor, was found to reduce the toxicity of irinotecan (CPT-11) in rats and humans. This study aimed to explore the pharmacokinetic interactions between these two compounds in rats and the possible underlying mechanisms.

Methods: Healthy male SD rats were randomized to receive a single dose of CPT-11 (60 mg/kg, i.v.) alone or in combination with thalidomide at 100 mg/kg by i.p. The plasma concentrations of CPT-11, SN-38 and SN-38 glucuronide (SN-38G) were analyzed by validated HPLC methods. In addition, rats were given thalidomide for five consecutive days before CPT-11 injection for multi-dose study. In vitro plasma binding, hepatic microsomal and metabolic inhibition studies in H4-II-E cells (a rat hepatoma cell line) as well as intracellular accumulation studies were also performed to explore the underlying mechanisms.

Results and Discussion: A single dose of thalidomide significantly increased the $AUC_{0\text{-}10h}$ of CTP-11 by 32.50% and its C_{max} by 19.36% (P<0.05), but decreased that of SN-38 by 24.58% (P<0.01). Similar results were observed with the multi-dose study. Thalidomide had no significant impact to the pharmacokinetics of SN-38G. Thalidomide and its hydrolysis products tested had no significant effect on the plasma protein binding of CPT-11 and SN-38, except for that thalidomide at 250 μM caused an increase in fu of CPT-11 by 6.7% ($P < 0.05$). Hydrolysis products of thalidomide (10 μM) decreased the CPT-11 (0.5 μM) hydrolysis by 16% ($P < 0.05$) in microsomes and thalidomide at 250 μM increased the conversion of SN-38 to SN-38G by 7.28% ($P < 0.05$) in H4-II-E cells. Thalidomide at 250 μM, PGA (a hydrolysis product of thalidomide) at 10 μM, or Thalidomide hydrolysis products (10 μM) increased the intracellular accumulation of SN-38 by 183.33%, 125%, and 200%, respectively (P<0.01).

Conclusions: The reduced toxicity of CPT-11 by the combination of thalidomide could be partially attributed to the pharmacokinetic interactions between these two drugs. Thalidomide hydrolysis products appeared to affect the hepatic metabolism of CPT-11 and SN-38 and intracellular accumulation of SN-38.

13) Therapeutic Drug Monitoring of Mycophenolic Acid in Renal Transplant Recipients in Singapore

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[2] *Department of Pharmacy, Singapore General Hospital*
[3] *Department of Renal Medicine, Singapore General Hospital*

Aim: To determine the optimal mycophenolate mofetil (MMF) dose in Asian renal transplant recipients (RTX), and to optimize therapeutic drug monitoring of mycophenolic acid (MPA).

Methods: Steady-state pharmacokinetics (PK) of MPA was performed in 47 stable RTX (63.8% male; age: 26-58years; body weight (BW): 33-108kg) receiving MMF (doses: 250-1000mg BD) for at least 3 months. All RTX were also on CsA (80-320mg/day) and prednisolone (5-18mg/day). This study was approved by the Ethics Committee and RTX recruited after written informed consent. Blood samples were collected at the targeted time-points of 0, 0.5, 1, 1.5, 2 and 6 hours (hr) after the morning MMF dose and plasma concentrations of total MPA determined using a validated HPLC assay. MPA PK parameters were calculated by non-compartmental methods. Stepwise multiple linear regression analysis was performed and validated to formulate limited sampling strategies for the estimation of the full area under the plasma concentration-time curve ($AUC_{ss,0-12}$) at steady-state.

Results: Mean (±SD) trough MPA (C_0) was 2.0±1.1mg/L; maximum MPA (C_{max}) was 13.8±7.2mg/L at T_{max} of 1.0±0.9hr. The $AUC_{ss,0-12}$ was 42.2±14.6mg·hr/L and dose-normalized $AUC_{ss,0-12}$ to 1g MMF was 73.1±33.3mg·hr/L. Drug exposure, as evaluated by the $AUC_{ss,0-12}$, demonstrated a weak but significant positive correlation with BW-adjusted MMF dose (r^2=0.33, $p<0.001$). An $AUC_{ss,0-12}$ of 45mg·hr/L could be attained with an MMF dose of 10mg/kg BW. MPA AUC was optimally estimated by a three time-point model ($AUC=2.5+C_{0.5}+3.0C_2+5.6C_6$; r^2=0.92); this formula demonstrated good precision with 88% of the AUCs being predicted with a prediction error within ±15% range.

Conclusion: As drug exposure and BW-adjusted MMF dose are correlated, MMF may be dosed based on BW rather than a fixed dose regimen. A limited sampling strategy involving 3 time-points (0.5, 2 and 6 hr) is proposed as a practical alternative to extensive AUC monitoring in the clinical setting to monitor MMF therapy.

14) Resistance Profiles of Multidrug Resistance Associated Protein 4 to Anticancer Drugs

Zhang J, Tian Q, & Zhou SF

Department of Pharmacy, Faculty of Science, National University of Singapore

Objectives: Multidrug resistance associated protein (MRP4) is able to transport structurally diverse lipophilic anions such as folate, glutathione and methotrexate. Increased expression of MRP4 in tumor cells is associated with resistance to various chemotherapeutic agents. The aim of this study was to investigate the resistance profiles of MRP4 to several anticancer drugs including cyclophosphamide, ifosfamide, topotecan, rubitecan and 10-hydroxy-camptothecin in the absence and presence of various MRP4 inhibitors.

Methods: HepG2 cells were transfected with empty vector or MRP4 cDNA. Drug effects on exponentially growing HepG2 cells with empty vector or overexpressing MRP4 were determined using the 3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyltetrazonium bromide (MTT) assay. To check for the effect of depletion of glutathione (GSH) on MRP4-mediated drug resistance, the IC_{50} values were determined in the presence of the GSH synthesis inhibitor DL-buthionine-(S,R)-sulphoximine (BSO). The effect of several MRP4 inhibitors such as diclofenac, celecoxib and MK-571 were also examined.

Results and Discussion: Overexpression of MRP4 conferred significant resistance to all drugs tested in the 4- and 48-hr drug-exposure assays. In *MRP4* transfected HepG2 cells, the presence of BSO decreased the cytotoxicity of these drugs in both 4- and 48-hr exposure assay. Diclofenac, MK571, and celecoxib also partially reversed the resistance observed with cyclophosphamide, topotecan, rubitecan and 10-hydroxy-camptothecin. Cyclophosphamide, ifosfamide, topotecan, rubitecan and 10-hydroxy-camptothecin are highly likely the substrates for MRP4.

Conclusions: MRP4 may play an important role in tumor resistance to camptothecins and oxazaphosphorines.

Invited Speeches
&
Podium Presentations

MS TAN LAI LEE

1996, BSc (Pharmacy) Second Class Upper

Completed pre-reg training in Guardian Pharmacy in 1997 and worked as a retail pharmacist before joining Quintiles, an international CRO in 1998. Worked in various positions within the company including drug safety and regulatory services. Switched to clinical operations as a CRA shortly after and have been there since. Currently, the head of clinical operations for Quintiles, responsible for it's clinical operations in Singapore, Malaysia and Vietnam.

Jan 2005 - Current, Associate Director, Clinical Operations, Quintiles East Asia
Oct 2002 - December 2004, Clinical Operations Manager, Quintiles East Asia
July 2001 - September 2002, Clinical Team Leader, Quintiles East Asia
July 2000 - June 2001, Senior Clinical Research Associate, Quintiles East Asia
July 1998 -June 2000, Clinical Research Associate, Quintiles East Asia
May 1998 - November 1998, Drug Safety/Regulatory Services Associate, Quintiles East Asia
July 1997 - April 1998, Registered Pharmacist, Guardian Pharmacy
June 1996 - June 1997, Pre-Registration Pharmacist, Guardian Pharmacy

Synopsis:
"Clinical Research as a Career Option" - Challenges and Opportunities for Pharmacists in Clinical Research
The talk will cover briefly, an overview of the Drug Development Process and the Clinical Research Industry, including the local industry players. It will touch on career options within the industry and in depth, job description and essential qualities for entry positions in the industry for pharmacists looking to venture into it.

DR MALINI OLIVO
Principal Investigator
Biophotonics Laboratory & Photodynamic Diagnosis and Therapy Laboratory , National Cancer Centre
Adjunct Associate Professor
National University of Singapore
E-mail: dmsmcd@nccs.com.sg

A/Pof Malini Olivo is currently the Principal Investigator of the Photodynamic Treatment and Diagnosis and Biophotonics Laboratories in the Singapore National Cancer Centre and SingHealth Research Facilities. and she holds an Adjunct appointment in the Department of Pharmacy at the National University of Singapore. She completed her Ph.D. in Bio-Medical Physics from the University College London and University of Malaya in 1990. Later, went on to do her Post-Doctoral Research fellowship at the British National Medical Laser Centre, University College London, UK and also at McMaster University, Hamilton and Toronto University in Ontario, Canada. She embarked on her research career in the Department of Radiology, McMaster University, Hamilton, and Princess Margaret Cancer Hospital in Toronto, Ontario, Canada before coming to Singapore to work in the Singhealth research campus in 1996.
A/Prof Malini Olivo has pioneered the area of clinical application of Photodynamic Diagnosis and Treatment in Cancer in Singapore. Her research interests include Photobiology and Photomedicine and also Biophotonics Imaging in confocal microscopy, spectroscopy and endoscopy .

Synopsis
Optical technologies using laser spectroscopy and imaging offer the ability to non-invasively diagnose and monitor early cancers in-vivo. Optical biopsy based diagnosis can be achieved in real time using automated techniques. Fluorescence imaging and spectroscopy using novel photosensitizers offer particular promise for the early diagnosis and treatment of cancer. Autofluorescence spectroscopy and imaging of endogenous fluorescent markers and/or drug induced fluorescence imaging offer a means of assessing both the structural and the biochemical progression of early disease. Clinical studies at the NCC have achieved promising results in the bladder, oral cavity and cervix using both exogenous and endogenous photosensitizers as fluorescence based markers for cancer detection and therapy. It is conceivable in the near future that highly sensitive endoscopic and non-endoscopic tumour detection methods can be successfully complemented by online staging and grading, to fully justify the concept of ‘optical biopsy’ and provide a multi-modality approach to cancer diagnosis and treatment.

A/P LAWRENCE K. NG

1984 University of Wisconsin at Madison B.S., Pharmacy
1988 University of Wisconsin at Madison M.S., Pharmaceutics
1991 University of Wisconsin at Madison Ph.D., Pharmaceutics

Herbal medicine as a treasure trove – a Poria Cocos story

The failure to control cancer deaths from common epithelial malignancies provides the ultimate rationale for developing new and effective anti-cancer agents for prevention and/or treatment of cancers. Asian diets and traditional Chinese medicines incorporate an extensive array of plant-derived foods, herbs and herbal extracts. Although much evidence exists that herbal components are linked to cancer prevention, the specific ingredients and sites of action remain elusive. In this short presentation, I will briefly discuss our attempts at characterizing the chemical composition of alcoholic extracts of *Poria cocos* and the anticancer effect of one specific component, pachymic acid. This seminar is designed to promote students' awareness and their interest in chemoprevention research.

Dr. Ng is an Associate Professor of Pharmacy, Department of Pharmacy, National University of Singapore. Prior to joining the NUS community in September, 2005, Dr. Ng was an Associate Professor of Pharmaceutics, School of Pharmacy, University of Colorado Health Sciences Center. His research focuses on the utilization of tissue culture as well as animal models to study problems associated with drug transport across the blood-brain barrier (BBB) and the development of novel methods of drug delivery targeted to the CNS. Recently, his laboratory has developed interests in the study of Chinese herbal medicines for chemoprevention of cancers. In that regard, efforts are directed at identification of novel herbal anticancer/chemopreventive agents and elucidation of their mechanisms in cell culture and animal models.

MR GWEE PAI CHUNG

07/2001-Present	National University of Singapore. Postgraduate, Department of Biochemistry, Faculty of Medicine. Thesis (in preparation): Genetic characterization of nucleoside analogue transporters *ABCC*4 and *ABCC*5 gene loci Supervisor: Assistant Professor Caroline Lee Guat Lay
07/1999-07/2000	National University of Singapore. BSc(Pharmacy)(Hon.), 2nd Upper.
07/1996-07/1999	National University of Singapore. BSc(Pharmacy), Pass with Merit.

Title: "The Hitchhiker's Guide to Graduate School"

Synopsis: "This talk attempts to provide prospective graduate students with some guidance in their journey through graduate study, highlighting issues that may prepare them for the rigors of research life. Topics are random: from how I looked for my Ultimate Question to how I "Don't Panic!" when the chips are down. The intent of the talk is therefore to draw on my experiences as a graduate student in Department of Biochemistry, rather t

The Role of Activin System in Keloid Pathogenesis

Mukhopadhyay A , Khoo A, Philips D, Werner S, Chan SY, Lim IJ, Phan TT

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Keloid scars represent a pathological response to cutaneous injury. Many growth factors have been found to play a crucial role in keloid pathogenesis. Activin A, a dimeric protein and a member of the TGF-β super family has been shown to regulate various aspects of cell growth and differentiation in many tissues. *In vitro* studies suggest a role of Activin A in the repair of mesenchyme and possibly also the epithelium, thus a disruption in the control of Activin expression could lead to tissue fibrosis. Our aim is to study the role of Activin system in keloid pathogenesis.

Methods: Normal and Keloid fibroblasts were treated with rhActivn A (100 ng /ml) and TGF–β_1 (1 ng/ml, 10 ng/ml) and subjected to MTT Assay. The expression of extracellular matrix proteins by normal and keloid fibroblasts under activin A and follistatin stimulation was assessed by Western blotting. Conditioned media, from non co-culture and co-culture of normal and keloid fibroblasts and keratinocytes, were subjected to ELISA to quantify Activin A and Follistatin concentrations respectively. RNase protection assay was performed to investigate the Activin A mRNA expression in Normal, Hypertrophic and Keloid tissue. Normal and keloid tissue were analyzed to investigate localization of Activin A and Follistatin by immunostaining.

Results: rhActivin A increased the proliferation of normal and keloid fibroblasts significantly. Activin A up-regulated collagen, fibronectin, α-SMA and PCNA expression in normal and keloid fibroblasts. ELISA demonstrated a higher concentration of Activin A and Follistatin in conditioned media from keloid fibroblasts. A similar pattern was observed for secreted Follistatin levels in keratinocytes. However Activin levels were higher in normal keratinocyte conditioned media as compared to keloid keratinocytes. In addition, an increased mRNA expression was observed in Keloid and Hypertrophic scar tissues. Immunostaining of keloid and normal tissues showed an increased localization of Activin A in the supra basal epithelium of keloid tissue as compared to normal tissue.

Conclusion: The results underscore the importance of Activin system in keloid biology and suggest a key role of Activin A in keloid pathogenesis.

Feasibility study of formulating tocopherol succinate (TOS) into nanoparticles

Han H and Ho PCL

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Tocopherol succinate (TOS), a redox-silent synthetic analogue of vitamin E (VE), has been commonly used as an antioxidant and recently shown to selectively induce apoptotic death of malignant cells by destabilizing their lysosomal membranes. In order to exert its anti-neoplastic activity, it appears essential that TOS reaches the tumour cells intact. However, it is fully hydrolyzed by non-specific hepatic esterases upon ingestion. In addition, TOS has low bioavailability when administered orally due to its poor aqueous solubility. To overcome these problems, the present study proposes the encapsulation of TOS using lipid-coated nanoparticles made up of a 1:1 mixture of zwitterionic phosphatidylcholine (PC) and negatively-charged phosphatidylserine (PS).

Methods: A high performance liquid chromatography (HPLC) assay method was first developed to measure TOS quantitatively. The nanoparticles were prepared by a freeze/thaw method and characterized using photon correlation spectroscopy for size and size distribution, transmission electron microscopy (TEM) for nanoparticle morphology and Malvern Zetasizer for surface charge. Loading efficiency and release studies were also conducted to evaluate the efficacy of TOS-loaded nanoparticles as a controlled drug delivery system.

Results and Discussion: TEM images revealed that the nanoparticles were spherical with an approximate diameter of 20nm. The use of Malvern Zetasizer showed that surface charge was negative, indicating that hydrophobic portions of TOS were embedded within the oily core whereas negatively-charged succinyl groups were oriented outwards. From the release studies, it was found that TOS-loaded nanoparticles released their contents in a temperature-dependent manner, with greatest TOS release at 37ºC.

Conclusion: TEM images and results from Malvern Zetasizer clearly evidenced the formation of TOS lipid-coated nanoparticles.

Nanocapsules of Platinum Compounds: Evaluation of their Pharmaceutical & Cytotoxic Properties

Yap KL, Ho PCL

Department of Pharmacy, Faculty of Science, National University of Singapore

Objective: Strategies to cause selective accumulation of anti cancer agents in tumour cells may be beneficial in the treatment of cancer. This may be achieved using liposomal encapsulation of drugs or through a nanoparticulate system of drug delivery. The formation and cytotoxicity of carboplatin nanocapsules composed of three types of phospholipid in different proportions were explored. Carboplatin nanocapsules of DOPC:DOPE:DOPS composition caused significant increase in cytotoxicity on the human hepatoma cell line HepG2.

Methods: Carboplatin nanocapsules were prepared by hydrating dry lipid films with saturated drug solution and subjecting samples to ten freeze thaw cycles. Efficacy of nanoparticles in causing tumour cell death was compared to that of the free drug in HepG2 cell line. Release profile of drug from the nanoparticles was also illustrated using quantification by HPLC. Morphology was also noted using Transmission Electron Microscopy.

Results and Discussion: Carboplatin nanocapsules of the DOPC:DOPE:DOPS combination showed a significant increase in efficacy of killing the HepG2 cells. At 37ºC, such a combination allowed a gradual release of drug into the environment. The nanoparticles are thought to be composed of a solid drug core wrapped by the lipid layer, which may enable an increased entry of drug into the tumour cells. Such a mechanism may be possible as HepG2 cells have an abundance of LDL receptors which may cause the selective uptake of the particles.

Conclusion: Nanoparticulate drug delivery systems have the potential to modify drug therapy to enable a smaller amount of drug to be as efficacious. This lowered amount of drug used may reduce the many undesirable side effects of anti cancer therapy. Further studies would need to be done to elucidate the mechanism of increased killing and to formulate improved nanoparticles.

American Association of
Pharmaceutical Scientists

Announcing the
2nd AAPS-NUS Student Chapter Symposium

The venue for the 2nd AAPS-NUS Student Chapter Symposium will be
National University of Singapore
January 2006

Please check the AAPS-NUS Student Chapter web site for details on Symposia 2006

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