PROCEEDINGS OF
2ND
AAPS-NUS STUDENT CHAPTER SYMPOSIUM

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Faculty of Science
National University of Singapore
Republic of Singapore

AMERICAN ASSOCIATION OF PHARMACEUTICAL SCIENTISTS
NATIONAL UNIVERSITY OF SINGAPORE

Message from the Chair

Welcome to the 2nd 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.

Our 112 chapter members are from Pharmacy, Biochemistry, Biology, Life Science, Singapore-MIT Alliance (SMA), and NUS Nanoscience & Nanotechnology (NUSNNI), including undergraduate, postgraduate students and postdoctoral research scholars. In the near future we will also welcome student members from local polytechnics in Singapore.

The program for this meeting features 6 posters and 11 podium presentations by chapter members and invited speakers from academia and industry. We have the strongest support from GlaxoSmithKline (GSK) Pharm., as two speakers including Dr Alan Catterall, the Vice President and Managing Director will give very interesting and informative presentations for this symposium.

Best Presenter Awards will be given to the outstanding student 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 has made this meeting rewarding for all participants. Special thanks to Dr Shufeng Zhou of Department of Pharmacy, National University of Singapore for his continuous support to our biannual symposia.

Regards,
Lifeng Kang, Chapter Chair

AAPS-NUS Student Chapter Executive Committee 2005/06

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Podium Presentations

Challenges and Opportunities for Pharmaceutical Manufacturing and Development in Singapore

Dr Alan Catterall, Vice President and Managing Director, GlaxoSmithKline (GSK)

The Changing Face of Quality

Ms Koo Siang Chueng, Senior Compliance Officer/LeanSigma Expert, GSK

The concept of quality evolves with time. What was previously considered good quality may not be adequate in today's context. While advances in science and technology drive changes in regulatory requirements, moving regulatory goal-posts also propel the development of science and technology. These challenges change the requirement for scientists and create many new employment opportunities in the pharmaceutical industry.

Presentation outline

- What is Quality
- Why do we have regulatory authorities like FDA
- How have the regulatory expectations of Quality changed over time
- What is the current expectation and challenges

Liposome formulations of therapeutic antibodies: A journey toward the development of rational antibody/drug combination therapies for cancer

Gigi N. C. Chiu

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

In the combat of cancer, antibodies (Ab) have emerged as the most rapidly expanding class of pharmaceuticals. The therapeutic effectiveness of Ab can be attributed to multiple functional properties of these molecules, including ligand binding competition, Ab-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), interference with receptor dimerization and signaling, and induction of apoptosis.

Various strategies have been undertaken to increase the efficacy of Ab-based therapies, and among these the use of multivalent Ab or fragments against tumor antigens have generated a great deal of interest. When tumor antigens are exposed to multivalent constructs of Ab or fragments, clustering of the target/Ab complex occurs as a result of increased valency and avidity of the construct, which subsequently triggers various cellular signals including induction of apoptosis, inhibition of cell growth/survival, and/or internalization of the surface tumor antigen. Our strategy to develop multivalent Ab constructs is via grafting of Ab molecules onto liposome membrane surface. Using trastuzumab and rituximab as examples, enhancement in the Ab activity of up to 25-fold was observed when the Ab were presented in the multivalent liposome formulation. More importantly, the multivalent liposomal Ab constructs were able to down-regulate the active, phosphorylated forms of pivotal signaling molecules including Akt and NF-kB that mediate chemoresistance and cell survival.

Based on these observations, the use of liposome offers not only the advantage of an agent that enhances Ab activity but also the potential of co-delivering Ab/drug combinations that could sensitize cancer cells to drug-induced cytotoxicity and improve the responses to chemotherapy. What remains to be addressed in the development of liposomal Ab/drug combination products is to screen for combinations with optimal therapeutic effects and identification of pharmaceutically viable developmental processes for such formulations comprising two therapeutic agents.

An Investigation of Cellular Responses to Tetrafluoroethylcysteine-induced Intramitochondrial Dysfunction

Dr Han Kiat Ho, Center for Molecular Medicine, A-STAR

Several disease states and chemical-induced toxicities are mediated through an early mitochondrial dysfunction. A better understanding of the molecular and biochemical events that transpires during such mitochondrial damage will help improve accuracy in preclinical screening of drug toxicities, and also in identifying potential drug targets for disease modulation. The studies described herein employ S-(1,1,2,2,-tetrafluoroethyl)-L-cysteine (TFEC), a major metabolite of tetrafluoroethylene (TFE), which covalently adducts a select subset of mitochondrial proteins, and causes mitochondrial stress. Results of these studies revealed that early mitochondrial changes, including the inhibition of the TCA cycle and ATP production, trigger a series of biochemical events that transcend organellar boundaries. Subsequent cascade of events are relayed through various signal transduction pathways that affect nuclear transcriptional responses and the induction of a number of cytosolic heat shock proteins (HSPs). An unexpected oxidative stress response, related to the activation of the Nrf2 transcriptional pathway was discovered and characterized. Overall, the results support an important role for mitochondrial dysfunction in the progression and outcome of some chemical-induced toxicities. Multiple signaling pathways can emerge from a focal subcellular lesion, leading to a plethora of targeted cellular responses in various organelles.

Development of Immunotoxins for Targeted Cancer Therapy

Koji Kawakami, M.D., Ph.D.
Department of Advanced Clinical Science and Therapeutics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

The progress of bioinformatics approach to understand the characterization of cell-surface antigens or receptors on tumor cells has stimulated the challenges to generate novel cancer vaccines or neutralizing monoclonal antibody therapeutics. Among the targeted cancer therapeutics, biologicals with targetable antibodies or ligands conjugated or fused to toxins or chemicals for direct cell-killing ability have been developed over the last two decades. These conjugated or fused chimeric proteins are termed immunotoxins or cytotoxins. Two targeted cytotoxins, DAB389IL-2 (ONTAKTM) targeting interleukin-2 receptors and CD33-calicheamycin (MylotargTM), have been approved by the FDA for cutaneous T-cell lymphoma (CTCL) and relapsed acute myeloid leukemia (AML), respectively. Immunotoxins including IL13-PE38QQR are being tested in the Phase III clinical trial in the United States. Immunoliposome is a liposome containing therapeutically active molecule that is conjugated to ligand or antibody on the liposomal surface to allow specific binding to the target cells. To develop new immunoliposomal targeted drugs, we are currently in the process of establishing a new laboratory in Biopolis, Singapore in collaboration with Oxygenix, Co., Japan.

BIOGRAPHY

Koji Kawakami, M.D., Ph.D., is an Associate Professor of the Department of Advanced Clinical Science and Therapeutics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan. After his training as a head and neck surgeon in Japan, Dr. Kawakami joined the Center of Biologics Evaluation and Research (CBER), United States Food and Drug Administration (FDA) and conducted a number of research projects in cytokine immunobiology, translational research, gene therapy, and targeted cancer therapy. Serving as a regulatory product reviewer specialized in tumor vaccine and cancer gene therapy at the FDA, Dr. Kawakami also reviewed investigational new drug (IND) applications submitted from U.S. industries and academic and governmental institutes. Currently, Dr. Kawakami is conducting research projects focusing on targeted cancer therapy and regulatory science. He also serves as Adjunct Associate Professor of National University of Singapore (Pharmacy Dept.) and Venture Incubation Partner of the University of Tokyo Edge Capital Co., Ltd., etc.

3D-confocal microscopy and real time cellular uptake of hypericin in N-methyl pyrrolidone formulation for visualized photodynamic activity in human bladder carcinoma cells

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Objective: Hypericin (HY) is a promising potent photosensitizer found in St. John's wort to detect and treat cancer. Trafficking of HY across *in vivo* chick chorioallantoic membrane and the photodynamic activity were increased when N-methyl pyrrolidone (NMP) was incorporated in the vehicle (1,2). HY uptake was usually obtained by *in vitro* studies after incubation. There had been no reported direct real time investigation of HY uptake. This present *in vitro* study is the first to use 3D-confocal laser scanning microscopy (CLSM) to study real time dynamic sub-cellular localization of HY in a human bladder carcinoma MGH cells.

Materials & Methods: MGH cells were cultured and seeded before experiments. HY and fluorescein were prepared to 100 nM working concentration before adding to cells. Nontoxic NMP concentrations, 0.05 % and 1 % were used. Real time dynamic uptakes of drugs were carried out by CLSM. Cellular fluorescence was analyzed.

Results & Discussion: Fluorescein was used as a model as membrane penetrant. Cells treated fluorescein without NMP showed no fluroescein penetration whilst cells with NMP showed fluorescein uptake. This indicated NMP was able to improve membrane permeability. For cells treated with HY-NMP 1 % gave higher red HY intensity compared to cells treated with HY-NMP 0.05 %. The survival analysis revealed that there was a significant difference between treatments with HY-NMP 1 % and HY-NMP 0.05 %, indicating poorer survival for cells with 1 % NMP (RR = 0.498, 95 % CI = 0.355 − 0.697, $p < 0.0001$).

Conclusion: This study demonstrated the technique of using 3D-CLSM to study the real time cellular uptake of HY, in the NMP formulation. With the appropriate use of NMP, HY-NMP formulation can enhance the HY-PDT mediated anti-cancer efficacy.

1. C. L. Saw, P. W. Heng, W. W. Chin, K. C. Soo, and M. Olivo. Enhanced Photodynamic Activity of Hypericin by Penetration Enhancer N-Methyl Pyrrolidone Formulations in the Chick Chorioallantoic Membrane Model. *Cancer Lett* (in press, available on-line 28 July 2005).

2. C. L. Saw, M. Olivo, W. W. Chin, K. C. Soo, and P. W. Heng. Transport of Hypericin across Chick Chorioallantoic Membrane and Photodynamic Therapy Vasculature Assessment. *Biol Pharm Bull* **28**: 1054-60 (2005).

Effect of hydrophilicity and surface topography of nanostructured and transparent polymer membranes with thermosensitivity on cell proliferation

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Objective: We have previously reported nanostuctured and transparent polymer membranes with thermosensitivity for use as wound dressing or support for cell grafting, fabricated by polymerization of a bicontinuous microemulsion consisting of N-Isopropyl-acrylamide (NIPAAm), methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (HEMA), and the nonionic polymerizable surfactant ω-Methoxy poly(ethylene oxide)$_{40}$ undecyl α-methacrylate macromonomer (C_1-PEO-C_{11}-MA-40). Cell adhesion and proliferation on polymer substrates are influenced by the chemical and physical surface properties of the materials. In this study, the relative effects of membrane hydrophilicity and surface topography on the attachment and proliferation rate of primary human dermal fibroblasts and primary human keratinocytes over the surface of transparent polymer membranes were determined *in vitro*.

Methods: A series of transparent polymer membranes with reduced hydrophilicity was synthesized by reducing surfactant (C_1-PEO-C_{11}-MA-40) and 2-hydroxyethyl methacrylate (HEMA) concentrations. A second series of transparent polymer membranes with surface grooves of 5.0, 10.0, 15.0 and 20.0μm, separated by ridges of 5.0μm was fabricated on a silicon wafer to obtain the desired surface topography. Cell proliferation was quantified by carrying out Alamar blue assays.

Results and Discussion: Fluorescence quantification showed that neither the membranes with reduced hydrophilicity nor the membranes with surface topography result in a significant effect on the proliferation rate of both primary human dermal fibroblasts and primary human keratinocytes. However, it was observed that the two cell types respond slightly differently to the membrane compositions and surface topographical cues.

Conclusion: All together, the results showed that the extent of hydrophilicity reduction carried out in this study did not result in a significant improvement in the cell proliferation. Also, neither the presence of surface grooves nor the dimension of the grooves employed in this study result in a significant effect on the cell proliferation. Nonetheless, the normal proliferation and the healthy shape of both cell types demonstrates the potential of these membranes as effective substrates for the *in vitro* growth of human dermal fibroblasts and keratinocytes and as excellent candidates for the development as a cell grafting support.

Rapid Quality Surveillance of Pharmaceutical Ginseng Biomolecules

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Paper Category: Analysis and Pharmaceutical Quality (APQ)

Objective: Quality of pharmaceutical products like ginseng is important for ensuring consumer safety and efficacy. Many ginseng products sold today are in various formulations which render it less identifiable by smell, taste or physical appearance. Furthermore, as ginseng is expensive, adulteration with other cheaper products occurs. Hence quality assurance of ginseng is needed. Since different chemical constituents in herbs like ginseng tend to exhibit characteristic IR fingerprints, the aim of this study is thus to assess the feasibility of IR spectral wavelengths in the rapid quality surveillance of ginseng.

Methodology: Ginsenoside standards and ginseng root samples were analyzed using an FTIR spectrometer. Their IR spectra were compared with each other, as well as with adulterants and commercial products based on spectral similarity and chemometric modeling of the spectral data.

Results and Discussion: The species of ginseng can be differentiated from each other and from the morphological adulterants based on the techniques used in this work for both qualitative and quantitative analysis of their spectral fingerprints. An analysis of three commercial ginseng products is also successfully demonstrated based on this work. Additional findings will also be discussed to further show that traditional means of ginseng authentication based on morphology are hardly reliable, thus there is a need for other methods of ginseng authentication.

Conclusion: In conclusion, the results of the present study show that the opportunity of using infrared wavelengths for the rapid quality surveillance of ginseng is proven to be appealing.

Soft Skin Adhesive (SSA) Gel for Transdermal Delivery

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Objective: The purpose of this study was to investigate the feasibility of developing a transdermal drug delivery (TDD) system for haloperidol used in the maintenance therapy of psychosis. The effects of various drug loadings, vehicles and enhancers on the percutaneous absorption of haloperidol in a soft skin adhesive (SSA) gel across human epidermis were evaluated.

Methods: Permeation study was done using flow-through diffusion cell system at 37°C. The vehicles investigated were propylene glycol (PG) and isostearyl alcohol (ISA). The enhancers evaluated were 2-ethylhexyl salicylate and (+)- cedryl acetate. The concentrations of vehicles and enhancers were both at 5%w/w.

Results and Discussion: Flux obtained by non-linear regression using a statistical software gave the same conclusions as that obtained by manual plots. The flux of haloperidol was different depending upon the drug loading and vehicle used. The flux of haloperidol increased significantly as the drug loading increased from 1mg/g to 3mg/g or 10mg/g. The permeation rate of haloperidol was significantly higher in formulations containing PG than that without a vehicle. Incorporation of enhancers into the adhesive matrix, on the average, did not significantly modify the permeation rate of haloperidol.

Conclusion: The steady state flux obtained from the SSA gel containing 10mg/g haloperidol, 5% w/w PG and 5% (+)- cedryl acetate was predicted to be high enough to obtain therapeutic effect and to meet the targeted daily-permeated dose. Future studies are needed to ascertain the physical properties and the stability of the adhesive.

Effect of Gleevec in Keloids

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Objectives: Keloids are benign dermal fibroproliferative disorder characterized by uncontrolled synthesis of extracellular matrix (ECM) components in predisposed individuals which occurs after trauma, burns, surgery, inflammation and possible spontaneously. Though a wide variety of treatment has been used, recurrence remains a problem. Previous studies in the lab showed that Stem Cell Factor (SCF) is up-regulated in keloids. In this study, an attempt was made to investigate the receptor for SCF, which is KIT receptor and explore the effect of Gleevec against KIT receptor in keloids. In addition, effect of Gleevec on profibrotic markers for keloid was also examined.

Methods: Fibroblasts and keratinocytes were cultured in DMEM medium and Keratinocytes Growth Medium respectively until confluent at 37°C in a humidified atmosphere of 95% air and 5% CO2. Cell proliferation study was carried out using MTT assay with Gleevec exposure for 1, 2, 3, and 5 days in DMEM and 5% fetal calf serum (FCS). Western Blot analysis was used to examine the effects of Gleevec on ECM components of fibroblast and keratinocytes.

Results and Discussion: Serum was demonstrated to have a positive effect on the inhibition efficiency of gleevec in both normal and keloid fibroblasts and keratinocytes thus highlighting the selectivity of gleevec for active cells. In addition, Western Blot analysis showed significant decrease of alpha-smooth muscle actin (α-SMA), a phenotypic marker for myofibroblasts, and fibronectin (major component of ECM) in keloid fibroblasts.

Conclusion: Gleevec could be a potential therapeutic agent for keloids.

Magnetic target gene delivery

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Objective: A novel use of magnetic fields to deliver therapeutic drugs or DNA with the nanoparticles on cancer treatment. This is called magnetofection.

Method : Magnetic nanoparticles provided by Chemicell technology GmbH. To generate complexes: Add diluted DNA (serum free) to 2-4 mircogram per ml of nanoparticles. after 20 mins incubation, add 500 microliteres of complexes of this to cell. Position magnetic plate for upto 15 mins, measures the transfection efficiency, it can be done via GFP reporter gene.

Results and Discussion: Overexpression of GFP significantly short time in the cell lines conferred transfection after 20 mins compare insignifciant transfection ratio in control. More cell lines and time factor need to be investigated and explored. Further optimization is needed as well. Also we will proceed to in-vivo animal models.

Conclusion: We observed magnetic targeted delivery is a very promising area to explore however,we have to bear in mind on the toxicity and adverse effects can be contributed in the process. Therefore thorough study is need to be carried out on these as well.

NUS Nanoscience and Nanotechnology Initiative (NUSNNI)

NUS has launched the NUS Nanoscience and Nanotechnology Initiative (NUSNNI) to promote this area of research few years back. The aim of NUSNNI is to initiate and coordinate long-term nanoscience and engineering research. Its objective is to achieve fundamental discoveries of novel phenomena, processes and tools. NUSNNI will provide the necessary support to facilitate efforts by faculties, researchers and students interested in pursuing this area of research. The focus areas include: nanomaterials fabrications, nanofiber tecnology , nanobiotechnology, nanomagetics etc. Then application will be the area of Biotechnology, Medicine, Infocommunications and Engineering Sciences.

I am working within gene delivery group in DBS, as gene therapy is one of the seven key focus in NUSNNI. The mechanism of guided or targeted delivery could be ligand binding via polycationic coating particles or field induced drift. In general, this gene therapy can applied on cancer treatment or cell repair. Targeted gene delivery to selected cell types provides a means for highly specific gene expression. Improved efficiency of gene transfer could be achieved through enhancing the entry of gene vectors into the desired cells and reducing the uptake of the vectors by non-target cells. For example, our group members developing chimeric peptides containing a nucleic acid binding domain linked to a receptor-binding domain of neurotrophins to target gene delivery vectors to tumors cells. These and other functional peptides are also tried to be conjugated to cationic polymer-based DNA vectors. The developed materials would form stable nanoparticles with DNA complex that are suitable for in vivo targeting of cancer gene therapy. My research is focus on magnetic field induced physical interaction.

Posters

Thalidomide Attenuates Chemotherapy-Induced Intestinal Lesions Via Down-regulation of TNF-〈 and Abrogation of Intestinal Epithelial Apoptosis

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Objective: Cancer chemotherapy often causes severe intestinal damages which have been ascribed to increased pro-inflammatory cytokines production and epithelial death. In the present study, we tested the hypothesis that the increased intestinal TNF-α expression and intestinal epithelial apoptosis by chemotherapy could be suppressed by anti-TNF-α agent.

Methods: A rat model with intestinal damages was set up by intravenous (i.v.) injections of CPT-11 for four consecutive days. Thalidomide, an anti-TNF-α agent, was used in our study to antagonize CPT-11 induced intestinal lesion. Diarrhea, intestinal inflammation, cytokines including IL-1β, IL-2, IL-6, IFN-γ and TNF-α as well as intestinal epithelial cell apoptosis were monitored over the whole study (11 days).

Results and Discussion: Our results demonstrated that administration of CPT-11 resulted in severe diarrhea and histological damages, accompanied with increased TNF-α expression and intestinal epithelial cell apoptosis on days 5, 7, 9 and 11 in rats. Combination of thalidomide (100 mg/kg, intraperitoneal (i.p.) injection for eight consecutive days starting one day before CPT-11 injection) significantly attenuated diarrhea and histological lesion caused by CPT-11, accompanied by inhibition of TNF-α expression and intestinal epithelial cell apoptosis.

Conclusion: These findings suggest a potent inhibitory role of anti-TNF-α agent on chemotherapy-induced gastrointestinal toxicity via modulation of intestinal TNF-α production and intestinal epithelial cell apoptosis. This observation might be of therapeutic value for identifying new agents that alleviate chemotherapy-induced intestinal toxicity.

Pharmacokinetics of Recombinant Human Endostatin in Rats

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Objective: The pharmacokinetics of recombinant human endostatin (rh-Endo) has not been established in the rat, although this species of animal is commonly used in the study of rh-Endo. This study aimed to investigate the pharmacokinetics, tissue distribution, and excretion of rh-Endo in rats.

Methods: ^{125}I-radiolabeled rh-Endo was administered to healthy rats by i.v bolus injection at 1.5, 4.5 and 13.5 mg/kg.

Results and Discussion: The maximum plasma concentration and area under the plasma concentration versus time curve of rh-Endo increased proportionally with the increase of the dosage. There were no significant differences in total body clearance (CL) and elimination half-life ($t_{1/2\beta}$) of rh-Endo among the three dosages used. A 93.5% and 2.2% of the radioactivity was recovered in the urine and feces, respectively; whereas only 0.1% was excreted into the bile. rh-Endo was rapidly and widely distributed in the liver, kidneys, spleen and the lungs. Furthermore, a significant allometric relationship between CL, but not volume of distribution (V_d) and $t_{1/2\beta}$ of rh-Endo, and the body weight was observed across mouse, rat and monkey, with the predicted values in humans significantly lower than those observed in cancer patients.

Conclusion: rh-Endo exhibited a linear pharmacokinetics in rats and its major elimination route was urinary excretion.

Analysis of Oxazaphosphorine Anticancer Drugs' Sensitivity in HepG2 Cells Overexpressing MRP4

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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 oxazaphosphorine anticancer drugs including cyclophosphamide and ifosfamide 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. Other alkylating agents including melphalan, busulfan, nimustine hydrochloride and mechlorethamine hydrochloride were screened.

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

Conclusions: MRP4 may play an important role in tumor resistance to oxazaphosphorine anticancer drugs.

Compatibility Study of Small Molecule Gelling Agents (SMGA) Gels For the Skin Permeation of Haloperidol with Terpene Skin Penetration Enhancers

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Objective: Proper formulation is an important aspect of any dosage form design. A lack of compatibility between the active ingredient and excipients may induce unforeseen reactions and adversely affect the stability of the product. Small molecule gelling agent (SMGA) gels were developed as a matrix to deliver haloperidol through the skin. The objective of this work is to determine the most suitable gel formulation for transdermal delivery of haloperidol.

Methods: Gelators used are D-DBS 1 or 0.1% (w/v), or GP-1 5% (w/v). Solvents employed are propylene glycol (PG) and isostearyl alcohol (ISA). 5% (w/v) of citral or nerolidol was added as penetration enhancer. Either 3mg/ml or 10mg/ml of haloperidol was used. The study employed a four-factor half factorial statistical design to investigate the influence of factor level changes on the physical and thermal properties of each gel formulation with haloperidol over time. Gels were prepared by heating excipients at 120 ^{o}C for 2 hours before adding the drug and cooling under room temperature of 22 ± 1 ^{o}C. Clarity, homogeneity, colour and odour of the gels were assessed for potential physical changes. Thermal activity of samples was monitored using microcalorimetry.

Results and Discussion: All gels retained their respective clarity. Gelator, solvent and drug concentration choice influenced the formation of a clear or opaque gel. D-DBS, ISA based formulations exhibited incompatibility through a lack of homogeneity upon standing. Nerolidol, being odourless and colourless was a better choice of enhancer than citral. Thermal profiles of gels suggest that ISA may not be a suitable solvent in terms of larger heat production through interactions with other excipients.

Conclusion: D-DBS may not be compatible with ISA. A more opaque gel matrix may be preferred as haloperidol is light sensitive, hence favouring PG as a solvent choice. Further studies are to be done on drug concentration retention in the matrix.

Human Multidrug Resistance associated Protein 4 Confers Resistance to Camptothecins

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Introduction The multidrug resistance associated protein 4 (MRP4) is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family. MRP4 is able to transport cGMP/cAMP, nucleoside monophosphate analogs. Camptothecins (CPTs) have shown substantial anticancer activity against a broad spectrum of tumors by inhibiting DNA topoisomerase I, but tumor resistance is one of the major reasons for therapeutic failure. P-glycoprotein, breast cancer resistance protein, MRP1, and MRP2 have been implicated in resistance to various CPTs. In this study, we explored the resistance profiles and intracellular accumulation of a panel of camptothecins including CPT, CPT-11, SN-38, rubitecan and 10-OH-CPT and other anticancer agents in HepG2 cells with increased MRP4 expression.

Methods HepG2 cells were transfected with an empty vehicle plasmid (V/HpeG2) or human MRP4 (MRP4/HepG2). The resistance profiles of test drugs in exponentially growing V/HepG2 and MRP4/HepG2 cells were examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazonium bromide (MTT) assay with 4 or 48 h exposure time of the test drug in the absence or presence of various MRP4 inhibitors. The accumulation of CPT-11, SN-38, and paclitaxel by V/HepG2, MRP4/HepG2 cells was determined by validated high-performance liquid chromatography methods.

Results Based on the resistance folds from the MTT assay with 48 h drug-exposure time, MRP4 conferred resistance to CPTs tested. The resistance of MRP4 to various CPTs tested was significantly reversed in the presence of DL-buthionine-(S,R)-sulfoximine (BSO, a γ-glutamylcysteine synthetase inhibitor), MK571, celecoxib, or diclofenac (all MRP4 inhibitors). 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, whereas the addition of celecoxib, MK571, or BSO significantly increased their accumulation in MRP4/HepG2 cells. There was no significant difference in the intracellular accumulation of paclitaxel in V/HepG2 and MRP4/HepG2 cells, indicating that P-glycoprotein was not involved in the observed resistance to CPTs in this study. MRP4 also conferred resistance to cyclophosphamide and ifosfamide and this was partially reversed by BSO. However, MRP4 did not increase resistance to paclitaxel, carboplatin, etoposide (VP-16), 5-fluorouracil, and cyclosporine.

Conclusion Human MRP4 rendered significant resistance to CPT, CPT-11, SN-38, rubitecan, and 10-OH-CPT. CPT-11 and SN-38 are substrates for MRP4. Both cyclophosphamide and ifosfamide are potential substrates for MRP4. Further studies are needed to explore the role of MRP4 in resistance, toxicity, and pharmacokinetics of CPTs and oxazaphosphorines.

Stability of Haloperidol in SMGA Gels

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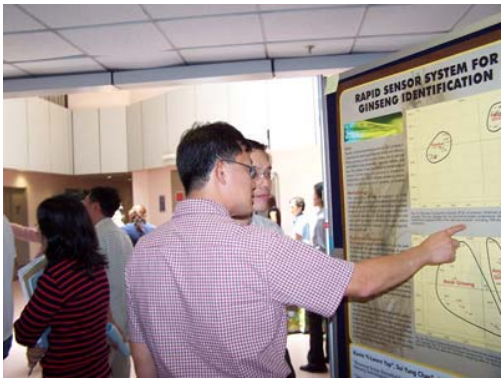
Objective: Stability of haloperidol in ISA-based SMGA gel when exposed to stress condition of temperature and light was investigated in this short-term stability study, with the aim and objective to identify the degradation kinetic model; to determine a tentative expiry date and an overview of it photostability profile.

Method: Sample of gel used for study consisted of haloperidol, and GP-1 in the concentration of 0.003% w/v and 5 %w/v respectively, dissolved in ISA under heat. For temperature study, triplicates of the samples were stored in temperature 60, 70, 80 and 90 °C (± 2 °C). Content of haloperidol were assayed using HPLC and expressed relative to content of haloperidol in samples stored at 5 °C. The data were then fitted to zero- and first-order degradation kinetic models and the adequacy of the model was evaluated through lack-of-fit test. Determination of tentative expiry period was made through the application of Arrhenius equation. For light study, triplicates of samples with thickness maintained at 25 mm were exposed to white fluorescent light; change in content haloperidol with time was compared with that of samples protected from light.

Results and discussion: Degradation of haloperidol in ISA-based gel under effect of temperature followed first-order kinetic reaction and determination of tentative expiry date from data from temperature stress study was not possible due to the inadequacy of the Arrehnius equation in describing the relationship of inverse of temperature and logarithm of degradation rate constant. Photostability study indicated that the translucent appearance of the gel did not confer extra stability to haloperidol against photodegradation.

Conclusion: The study can served as prior information for a better study design such that the tentative expiry period of haloperidol can be determined. Furthermore, the thermal stability profile of the drug in such system provides an indication that drug loss might be incurred even during the making of drug-gel preparation; hence a revisit to steps in the making might be essential to find the optimum conditions for the making of the preparation. No extra photostability is conferred to the drug by the gel appearance indicating that protection against light is essential to prevent drug loss through photodegradation.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