Proceedings

PharmSci@Asia

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Fudan University and Nanjing University

28-29 June 2007

Hosted by
School of Pharmacy, Fudan University

Level 1, Ming Dao Building, 130 Dong’an Rd., Fudan University
(Medical Campus), Shanghai, China 200032
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PharmSci@Asia

28 June 2007

School of Pharmacy, Fudan University
No. 130 Dong’an Rd, MingDao Building, Shanghai, China, 200032

PROGRAM

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08:30-08:50  Welcome Addresses
Prof Chen, Director of Foreign Affairs, Fudan University
Assoc Prof Chan Sui Yung, Faculty Advisor of AAPS-NUS Student
Chapter & Head of Department, Department of Pharmacy, National
University of Singapore
Prof Zhu Yi Zhun, Dean, School of Pharmacy, Fudan University
Stacey Anne May, M.A., Director, Public Outreach, AAPS (Video)

08:50-09:30  From Traditional Chinese Medicine to Scientific Chinese
Medicine: A Scientific Approach of Fudan's Experience
Prof Zhu Yi Zhun, Dean, School of Pharmacy, Fudan University

09:30-10:10  Micro- and Nanoscale Technologies for Tissue Engineering and
Drug Discovery Applications
Asst Prof Ali Khademhosseini, Harvard-MIT Division of Health
Sciences and Technology, Massachusetts Institute of Technology

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10:30-11:10  Transdermal Iontophoresis of Apomorphine – from in-vitro
Modelling to the Treatment of Parkinson Patients
Prof Hans Junginger, Visiting Professor at the Department of
Pharmaceutics, Faculty of Pharmaceutical Sciences, Naresuan
University, Phitsanulok, Thailand and at the Department of Pharmacy
at the National University of Singapore

11:10-11:40  Development of Functionalized Carbon Nanotubes as Delivery
Systems
Asst Prof Giorgia Pastorin, Department of Pharmacy, National
University of Singapore
11:40-11:55  Transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer as an efficient brain-targeting gene delivery vector  
Ms Rong-Qin Huang, Postgraduate student, Fudan University

11:55-13:30  Group Photo + Lunch + Poster viewing

13:30-14:00  Bioactive Constituents from some Chinese Medicinal Plants  
Prof Aijun Hou, Department of Pharmacognosy, School of Pharmacy, Fudan University

14:00-14:30  Predicting Druggable Proteins from Amino Acid Sequence by a Machine Learning Approach  
Assoc Prof Chen Yu Zong, Department of Pharmacy, National University of Singapore

14:30-15:00  Cell Patterning with Water Evaporation  
Dr Kang Lifeng, Postdoctoral Fellow, Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology and Overseas Postdoctoral Fellow, Department of Pharmacy, National University of Singapore

15:00-15:15  Bis-(-)-nor-meptazinols as Novel Nanomolar Cholinesterase Inhibitors with High Inhibitory Potency on Amyloid-β Aggregation  
Ms Xie Qiong, Postgraduate Student, Fudan University

15:15-15:30  Strategy to Prevent Matrix Lamination at Gastric pH via Formation of a Cross-linked Barrier  
Ms Ching Ai Ling, PhD Student, Department of Pharmacy, National University of Singapore

15:30-15:45  Tea Break

15:45-16:00  Effects of Different Polymer Types on Controlled Release of Endostatin Poly(lactic-co-glycolic acid) Microspheres  
Mr Wu Jinhui, PhD Student, Nanjing University

16:00-16:15  Identification of In Vitro Phase I Metabolites of Meisoindigo in Rat and Pig Liver Microsomes by LC-MS/MS  
Mr Huang Meng, PhD Student, Department of Pharmacy, National University of Singapore
16:15-16:30  Adsorption of Plasmid DNA onto N, N’-(Dimethylamino)ethyl-methacrylate Graft-Polymerized Poly-L-lactic Acid Film Surface for Promotion of in-Situ Gene Delivery
Ms Jiang Tingting, Postgraduate Student, Nanjing University

16:30-17:00  The Use and the Delivery of Natural Compounds and Derivatives for Cancer Therapy
Asst Prof Gigi Chiu Ngar Chee, Department of Pharmacy, National University of Singapore

17:00-17:30  Research in Pharmaceutical Technology and Formulation to Build Quality and Enhance the Performance of Drug Products and Processes
Assoc Prof Chan Lai Wah, Department of Pharmacy, National University of Singapore

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Assoc Prof Lawrence Ng Ka-Yun, Department of Pharmacy, National University of Singapore

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18:10-18:15  Closing Address
Ms Ching Ai Ling, Chair of AAPS-NUS Student Chapter

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29 June 2007
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Tour of Zhangjiang Campus

Zhang Heng Road, No. 825, Pudong New Area and Zhangjiang Hi-Tech Park, Shanghai, 201203.
ORAL PRESENTATIONS
Purification and Standardization of Chinese herbal extracts became a hot topic since last decade\(^1\). Though traditional Chinese medicine (TCM) has been used as a mixture from several herbs for centuries, it has been drawn much attention for studying the standardized Chinese herbs using modern technology\(^2,3\). Recently, we compared purified \textit{Salvia miltiorrhiza} extract (PSME) with Angiotensin-converting enzyme inhibitor, Ramipril, in \textit{in vitro} experiments and also \textit{in vivo} using animal model of myocardial infarction\(^4\). PSME was found to have a significantly higher trolox equivalent antioxidant capacity which indicated a great capacity for scavenging free radicals. PSME could also prevent pyrogallo red bleaching and DNA damage. After 2 weeks treatment with PSME or Ramipril, survival rates of rats with experimental myocardial infarction were marginally increased (68.2\% and 71.4\%) compared with saline (61.5\%). In another recent study, we evaluated the cardioprotective effects of PSME on myocardial ischemia/reperfusion injury in isolated rat hearts\(^5\) and in hypoxic vascular smooth muscle cells\(^6\). We found that PSME treated hearts showed significant postischemic contractile function recovery (develop pressure recovered to 44.2±4.9\% versus 17.1±5.7\%, \(p<0.05\); maximum contraction recovered to 57.2±5.9\% versus 15.1±6.3\%, \(p<0.001\); maximum relaxation restored to 69.3±7.3\% versus 15.4±6.3\%, \(p<0.001\) in PSME and control group respectively). Significant elevated in end-diastolic pressure, which indicated LV stiffening in PSME hearts might be resulted by exceed dose of PSME used. Purified and standardized Chinese herb could provide an alternative regimen for the prevention of ischemic heart disease.

References:
Micro- and nanoscale technologies can be used to study the interactions between cells and their surroundings as well as miniaturize assays for cell-based screening. In our lab we have developed various approaches at the interface between materials sciences, engineering and biology to control and study the interactions of cells with their microenvironment including the surrounding matrix, soluble factors and other cells. In addition, we will present work in the use of such technologies to miniaturize assays for screening applications. Examples include the fabrication of cell arrays comprised of various cell types, viruses and bacteria as well as biomimetic systems based on microengineering cell-cell interactions. Furthermore, we will describe 3D microfluidic systems that can be used in drug discovery.
Optimal drug delivery will remain a challenge also in the 21st century. Optimal drug delivery means that a drug is delivered in the right dose at the right time at the right place without inducing toxic side effects. Although a lot of progress has been made to achieve these goals, drug delivery is still far away from being optimal at the current time both with respect to reproducibility and predictability not to mention at all the desirable “drug delivery on demand” which means optimised drug delivery depending on the state of disease.

However, drug monitoring of the achieved drug levels is still far away from reality. Transdermal iontophoresis, i.e. the electrically driven transport of charged drug molecules by means of a mild current across human skin may be able to overcome this problem for special diseases. Iontophoresis of apomorphine, a potent anti-Parkinson disease drug with poor oral bioavailability was firstly done in vitro using a specialized diffusion cell with two electrodes which is able to mimic the conditions at the skin in vivo. The results showed the feasibility of high drug input into the skin especially in combination with suitable chemical penetration enhancers without causing serious skin irritations so that in vivo studies firstly in human volunteers could be realized. Going into the clinic we showed the ability to achieve therapeutic drug levels in Parkinson patients. Disease parameters such as rigor (stiffness) and tremor (trembling) can be monitored by suitable chips. Feedback with those signals with the computer of the iontophoretic delivery system may result in optimal disease treatment by drug input on demand avoiding toxic drug levels.

Although a lot of research has been done to develop therapeutic transdermal systems for therapeutic use, the achievements so-far have been disappointing. In 2006 the first simple iontophoretic systems with lidocaine as drug for local anaesthesia was finally approved by FDA. ALZA also has now FDA approval for commercialization of an iontophoretic fentanyl patch for the pain management in cancer patients.

Some references
BIOACTIVE CONSTITUENTS FROM SOME CHINESE MEDICINAL PLANTS

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Moraceae is a large family comprising sixty genera with approximately 1400 species, including some important groups such as Artocarpus, Morus, Ficus, and Cudrania. Artocarpus species are evergreen trees distributed over tropical regions of Asia and some members are used as traditional folk medicines in Indonesia, Thailand, Sri Lanka, and China.1 Previous research work on this genus revealed various isoprenylated flavonoids and a limited number of stilbenoids with structural and biological interests.2,3 Recently, a variety of isoprenylated flavonoids, stilbenes and their novel biogenetic derivatives, and arylbenzofurans, have been isolated from some Chinese Artocarpus plants by our group. Their antitumor activities were evaluated and the biogenetic pathway of some novel compounds was discussed.4,5

Cudrania species, a rich source of isoprenylated xanthones, have been investigated phytochemically and biologically.6 Cudrania tricuspidata (Carr.) Bur. is a deciduous shrub or tree distributed over China, Korea, and Japan. Its roots are applied in clinic for the treatment of digestive apparatus tumor, especially gastric carcinoma,7 and are also used as traditional Chinese medicine (TCM) “Chuan-po-shi” together with the roots of C. cochinchinensis (Lour) against gonorrhea, rheumatism, jaundice, boils, scabies, bruising, and dysmenorrhea.8 However, the anti-tumor principles have been unknown. Guided by the bioassay, 29 isoprenylated xanthones and flavonoids were isolated and identified.9 The structure-activity relationship was hypothesized. These results clarified the antitumor principles of C. tricuspidata, which are of important significance for the research and development of new drug candidate.

References
[8] Jiangsu New Medical College. Dictionary of Traditional Chinese Medicines; Shanghai Science and Technology Publisher: Shanghai, 2000; p 1731.
DEVELOPMENT OF FUNCTIONALIZED CARBON NANOTUBES AS DELIVERY SYSTEMS

Alberto Bianco, a Wei Wu a, Maurizio Prato b and Giorgia Pastorin a,c,*

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Introduction

Recently, carbon nanotubes (CNT) have attracted the attention of several scientists, due to their unique and intriguing structure and properties. These nano-objects seem to hold a lot of promises in biomedical applications for different reasons: inducible water solubility, high stability of the dispersion, lack of intrinsic immunogenicity and an efficient loading capacity.1

Experimental

It is known that the treatment of CNT under oxidative conditions (for example, sonication in mixtures of sulfuric and nitric acids) can cut the tubes and provides abundant carboxylated sites along their sidewalls. To achieve a double orthogonal CNT functionalisation, oxidized CNT were activated as acid chloride and reacted with phthalimide mono-protected diaminotriethylene glycol. This protecting group is particularly useful since it is highly stable to harsh acidic conditions and orthogonal to the Boc group, which can be subsequently introduced via the 1,3-dipolar cycloaddition to the sidewalls of CNT (Scheme 1).2,3

Scheme 1: a) Neat (COCl) 2; b) Pht-N(CH2CH2O)2-CH2CH2-NH2, dry THF, reflux; c) Hydrated NH2-NH2, EtOH, reflux; d) FITC, DMF; e) HCl 4m in dioxane; f) Fmoc-AmB, HOBt/EDCHHC/DEPA, DMF, 25% piperidine in DMF. BOC=tert-butyloxycarbonyl; DIPEA=diisopropylethylamine; DMF=dimethyl formamide; EDC=N-Ethyl-N’-(3-dimethylaminopropyl)-carbodiimide; FMOC=fluorenylmethyloxycarbonyl; FITC=fluorescein isothiocyanate; HObt=1-hydroxybenzotriazole; Pht=phthalimide group.
We initially removed the phthalimidic group, using a solution of hydrazine in ethanol, and the free amino group was coupled with fluorescein isothiocyanate (FITC). Then, Boc was cleaved using 4M HCl in dioxane and AmB was covalently linked to the amino group.

The aim of this work was first to explore a new strategy for the double functionalization of CNT and second to assess the characteristics of toxicity and uptake of CNT functionalised with AmB and fluorescein towards mammalian cells. To assess the biological properties of the novel doubly functionalized carbon nanotubes, we studied the toxicity effects of CNT on mammalian cells following its capacity to cross the cell membrane.

**Results and discussion**

The conjugation of AmB to CNT clearly reduces the toxic effects of the antibiotic on mammalian cells. At the highest doses, more than 40% of the cells died in the presence of AmB, whereas all the cells remained alive upon treatment with CNT. Furthermore, cell uptake of CNT was very fast as maximum fluorescence was observed after only 1h of incubation.

Finally, we evaluated the antifungal activity of CNT, functionalized with AmB, against 5 species of fungi. It was observed that the activity of the drug was not prevented by its covalent binding to CNT. Interestingly, conjugated AmB is definitely more potent than the free drug. The reason for this might be an increased solubility of the drug by conjugation to CNT. Alternatively, the binding to CNT and the presence of multiple copies of AmB per CNT molecule might favor the interaction of the drug with its target, the fungal membrane.

**Conclusion**

In summary, we were successful in preparing carbon nanotubes containing both fluorescein and amphotericin B. Our studies reveal that AmB covalently linked to carbon nanotubes is uptaken by mammalian cells without presenting any specific toxic effect. Therefore, multifunctionalised carbon nanotubes can be envisaged for the delivery of antibiotics to different types of cells by selective transport through the membrane. Finally, the covalent linkage of different drugs to carbon nanotubes is an approach that may be used to modulate the therapeutic action of the agent, thus obtaining new conjugates with interesting properties.

**References**

Introduction

Target identification and validation is an important first step in drug discovery processes. Despite increasing levels of spending and extensive use of new technologies, fewer innovative targets are emerging. Therefore new and improved methods and integrated molecular- and systems-based approaches are being explored for target discovery. The commonly used computational methods have primarily been based on similarity, domain affiliation, and structural studies, which tend to become less effective for targets that exhibit no or low homology to known targets and proteins with available 3D structures.

Hence methods independent of sequence and functional similarity and structural availability are needed. One machine learning method, support vector machines (SVM), has recently been explored for predicting druggable proteins from sequence-derived physicochemical properties irrespective of sequence similarity to known proteins. Druggable proteins are proteins that can be regulated by drug-like molecules, and disease relevant druggable proteins become targets.

Experimental

One strategy for predicting druggable proteins from their sequences without sequence similarity is to predict these proteins by using a sequence-independent classifier generated from the analysis of known targets that share common druggable characteristics but may be significantly different in sequence, structure and function. Proteins can be divided into druggable and non-druggable classes. Thus two-class classification machine learning methods such as SVM can be applied for developing an artificial intelligence model to separate druggable and non-druggable proteins.

Each protein is represented by a feature vector composed of sequence-derived descriptors representing its structural and physicochemical properties. SVM classifies proteins by projecting their feature vectors into a multi-dimensional space in which druggable and non-druggable proteins are separated by a hyper-plane.

Results and discussion

The performance of SVM for predicting druggable and non-druggable proteins was evaluated by a 5-fold cross validation study of 1,484 druggable and 6,637 non-druggable proteins. The computed prediction accuracies for druggable proteins P+ and non-
druggable proteins are in the range of 80.8%–88.2% and 95.5%–97.2% respectively. The overall accuracy is in the range of 93.1%–95.6%.

The capability of SVM was further assessed by comparison of the numbers of SVM predicted druggable proteins in the complete genome of H. sapiens, S. cerevisiae, C. elegans, D. melanogaster, C. albicans, M. tuberculosis, H. influenzae, and H. pylori with those estimated by other studies. Most of the encoded protein sequences in these genomes are not in the SVM training and testing sets. The numbers of SVM predicted druggable proteins are highly consistent with the estimated numbers of targets or druggable proteins from other studies.

Moreover, the performance of SVM was compared with that of a druggable domain affiliation (DDA) method based on a test of 35 published successful, research, and proposed research targets of Mycobacterium tuberculosis genome. DDA predicts druggable proteins by evaluating whether a member of the Interpro domain family of the studied protein is bound by a Lipinsky’s rule-of-five compound. 54% of all of the 35 targets and 85% of the 20 successful and research targets were predicted as druggable by SVM, which is substantially better than the percentages of 31% and 45% derived from DDA.

**Conclusion**

Statistical and proof-of-concept tests consistently show that SVM is useful for facilitating the identification of druggable proteins from sequence data. In addition to the incorporation of newly discovered knowledge and information into SVM and other in silico methods, target discovery can be further improved by collective analysis of multiple sequence, structure, systems and physiological profiles particularly sequence and functional similarity to known targets, drug-binding domain family affiliation, geometric and energetic features of protein structures, ligand-protein inverse docking and systems-related properties. These methods may potentially be developed into useful tools for facilitating the identification of novel targets.

**References**

CELL PATTERNING WITH WATER EVAPORATION

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Introduction
The extracellular matrix (ECM) can be studied by microscale technology[1]. Cell patterning over microwells can be achieved by various methods[2,3]. However, the patterning process requires specific facilities not readily available. In this study, a simple method was proposed to pattern the cells into microwells by water evaporation for high throughput screen study using cell microarrays.

Experimental

1 Prepare the microwells by UV-crosslinking
1) Plasma-clean the glass slides and cover glasses; 2) The slides were spin-coated with TMISA; 3) Prepared microwells with PEGDA 200 with 0.5% of the photoinitiator; 4) The prepolymer solution was placed onto a glass slide plasma-cleaned between two cover glasses and another cover glass on top; 5) With the photomask on top, subject to the UV light to cure the gel for 1 seconds; 6) The cover glass was carefully removed and the microwells were left attached; 7) Plasma-clean the wells to increase their hydrophilicity. Because surfaces of glass were hydrophilic while PEG was hydrophobic.

2 Water evaporation from the microwells
1) Prepare fluorescein sodium solution of 100 µg/ml; 2) Dip the microwells into the solution; 3) Pictures were taken every 30 seconds till each cell dries up, i.e., the green color disappeared completely.

3 Cell patterning with water evaporation from microwells
1) Microwells with diameter of 400 µm were prepared with TIMSA coating; 2) The microwells were plasma-cleaned for 5 min. Then a drop of cell suspension was added to the center of the microwells and spread evenly on the microwells to form a thin film; 3) Water will evaporate and the cell suspensions will shrink into the microwells because of hydrophobicity of the bottom of the microwells.

4 Live/dead test
1) Live/dead test were conducted. Two recognized parameters of cell viability—intracellular esterase activity and plasma membrane integrity—were tracked. Live cells fluoresced green, showing intracellular esterase activity that hydrolyzed the fluorogenic esterase substrate (calcein AM) to a green fluorescent product, and dead cells fluoresced red, their plasma membrane being compromised and therefore permeable to the high-affinity, red fluorescent nucleic acid stain (ethidium homodimer-1); 2) Immediately after the cells were seeded into the microwells, a drop of live/dead testing solution was added to the microwells and wait till the water shrinks into the microwells. The microwells were then visualized under the microscope.

Results and discussion
Figure 1 Water evaporation from microwells. The diameter of the well is 400 µm

Figure 2 Cell patterning with water evaporation. The diameter of the well is 400 µm.

It was shown in Figure 1 that the average time was 6.33 ± 0.61 minutes for the microwells to dry up. When the microwells were seeded with cells, the average time increased to about 8 minutes, because of the cells which retained water (Figure 2). It was also shown that most cells survived for more than 10 minutes. The cells docked into the microwell accurately due to the hydrophilic difference between the hydrophilic microwell bottom and the hydrophobic microwell walls. With water evaporation and shaking, cells were collected and retained by the microwells. This is an important step in high throughput screening study for ECM with microarrays.

Conclusion
Cells can be seeded into microwells with water evaporation.

References
THE USE AND THE DELIVERY OF NATURAL COMPOUNDS AND DERIVATIVES FOR CANCER THERAPY

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Introduction

Appropriate combinations of anti-cancer agents that target and attack the survival mechanisms would yield great therapeutic potential, and this includes the use of naturally occurring anti-cancer compounds and their derivatives. However, compounds with potent anti-cancer activities often have bulky hydrophobic groups within their chemical structures, rendering them poorly water-soluble. There are two main interests of my research group: 1) to explore the use of naturally derived compounds in single and combination treatment regimes for cancer, and 2) to improve the water solubility of these compounds using lipid-based drug delivery platforms. The ultimate goal is to deliver synergistic, anti-cancer drug combinations to simultaneously attack various survival mechanisms of the cancer cells.

Experimental

A number of cellular and molecular techniques are used to test the anti-cancer activity of the natural compounds and derivatives as well as to elucidate the molecular mechanism(s) of action in breast and ovarian cancer cell lines. These include cell viability assay, flow cytometry-based cell cycle analysis and apoptosis assay, and western blotting. To assess whether the natural compounds or their derivatives would have any synergistic effect with conventional chemotherapeutic drugs, combinations of these two drug groups are evaluated in cancer cells lines, and the median effect principle, developed by Chou and Talalay, is used to analyze the drug combination effect. To increase the water solubility of these natural compounds, lipid-based nanoparticulate technology, including liposomes and micelles, is used as the platform to achieve optimal formulations of these natural compounds.

Results and discussion

Thus far, my research group has tested the effects of triptolide, quercetin, genistein, and safingol as single agents or in combination with chemotherapy. Our in vitro studies showed that these compounds are effective in breast and ovarian cancers. The cytotoxic effects of conventional anti-cancer drugs such as doxorubicin, gemcitabine and 5-fluorouracil could be enhanced when used in combination with quercetin or safingol. Liposome formulations of quercetin and triptolide have been developed to increase water solubility of these compounds, and the activities of these formulations are currently under study.
Conclusion

Natural products are rich in anti-cancer compounds which contribute to the drug discovery and development for cancer therapy. Lipid-based drug delivery platform is a viable approach to increase the water solubility and delivery of these anti-cancer drug compounds as single agents or in combinations with conventional chemotherapeutics.

References

Pharmaceutical companies spend more than US$800 million on the average to get a new drug approved. This phenomenal investment includes numerous studies to ensure that the drug is efficacious and safe. Many potential drugs have fallen by the wayside, either because they do not perform up to expectation or they cause unacceptable side-effects. It has been reported that only about one in 17 candidate drugs identified finally made it to the market. If the drug being researched fails in the late stage of the development process, the losses will be tremendous and cause financial ruin to the company.

Due to the high stakes involved in the search for new drugs, it is wise to explore other methods which can help to enhance the performance of existing drugs and extend the product line. This is where pharmaceutical technology and formulation can play important roles. A drug substance can be developed into products of different forms with modified properties by varying its formulation and/or method of processing. For example, the drug can be formulated as a tablet or solution for oral administration; or as a gel for topical application. Its therapeutic activity can be modified by incorporating certain additives to the formula. Its side-effects can be reduced and its release can be controlled by coating the drug or encapsulating it in a polymeric matrix. A good knowledge of pharmaceutical technology and formulation therefore provides tremendous flexibility to the improvement of drug products and extend the product line.

GEA-NUS is one of the research laboratories established in the Department of Pharmacy, National University of Singapore. Its mission is to advance pharmaceutical science and technology, nurture competent professionals and assist pharmaceutical companies to maintain and improve their competitive edge in the global market. Over the years, GEA-NUS has conducted extensive research to gain a deeper understanding of pharmaceutical materials, processes and equipment. In collaboration with numerous parties, its research interests include pre-formulation and material characterization, pharmaceutical processes such as granulation, spheroidisation, pelletisation, microencapsulation, coating and extraction. These research interests have extended to the study of transdermal and pulmonary besides oral delivery systems. In line with the current interest of the pharmaceutical industry, GEA-NUS is now actively involved in the development of process analytical technology (PAT) to build quality into manufacturing processes. PAT studies undertaken include in-line sizing, NIR blending, on-line imaging of materials during processing.
From earlier investigations, GEA-NUS has developed a model based on artificial neural network (ANN) and data clustering to predict the performance of microcrystalline cellulose in extrusion-spheronisation. It has also successfully employed the coating and microencapsulation technology to control drug release, preserve drug stability and enhance drug performance. These studies have resulted in the design of better drug carriers for pulmonary delivery, small particles with uniform coats for various applications, norcantharidin-loaded microspheres for chemoembolization, alginate-starch microspheres for encapsulation of PUFA, lipid microspheres for moisture-sensitive drugs and *Saccharomyces cerevisiae*-loaded microspheres as bioreactors for alcohol production. The extensive investigations of pharmaceutical processes have enabled GEA-NUS to provide invaluable recommendations for the design of pharmaceutical equipment. This has helped to forge a strong partnership with the equipment manufacturers.

GEA-NUS takes pride in advancing pharmaceutical science and technology and nurturing competent professionals for the industry. The research programme and highlights of GEA-NUS in pharmaceutical technology and formulation will be presented at the symposium.
Natural products have played a critical role in the discovery of biologically-active small molecules that have either become therapeutic agents themselves, or have provided the template for such therapeutic agents. Although herbal plants and diets are rich sources of natural products, they have not been properly exploited during past attempts to discover new compounds nor are they adequately examined in terms of their molecular targets and their relevance to disease treatment and/or prevention. Our work focuses on the use of chemical, biochemical, molecular biological, and animal model techniques for evaluation of various herbal and nutrition constituents and their potential applications in treatment and/or prevention of cancers and strokes. Specifically, we are seeking to promote treatment and prevention research that focuses on the identification and characterization of molecular targets for these naturally derived agents that are fundamental for the development of effective, tailored and preemptive strategies for reducing cancers and strokes in humans. This presentation will highlight some of our recent work to identify the active components in Poria cocos (a Chinese herb) and garlic that are responsible for their possible anti-tumorigenic effects.

This work was supported by a NIH Grant CA 115269 (to Ng KY) and a National University of Singapore Academic Research Fund (R148-050-068-101 and R148-050-068-133).
LACTOFERRIN-CONJUGATED POLYETHYLENEGLYCOL-MODIFIED POLYAMIDOAMINE DENDRIMER AS AN EFFICIENT BRAIN-TARGETING GENE DELIVERY VECTOR

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Introduction

The blood-brain barrier (BBB) poses great difficulties for gene delivery to the brain. Most current gene vectors do not cross the BBB following an intravenous administration, and must be given via craniotomy or intracerebral injection which are considered to be highly invasive and unable to deliver exogenous genes to global areas of brain [1, 2]. To circumvent this problem, we applied polyamidoamine (PAMAM) and lactoferrin (Lf) to design a novel brain-targeting carrier, PAMAM-PEG-Lf. PAMAM, a nanoscopic high-branching dendrimer, was used as the main gene vector; Lf, for the first time, was selected as a brain-targeting ligand; PEG, as a spacer, was applied to link PAMAM and Lf.

Experimental

Nuclear magnetic resonance (NMR) spectroscopy was employed to evaluate the synthesis of vectors. The characteristics and biodistribution of gene vectors were evaluated by fluorescent microscopy, flow cytometry, and a radiolabeling method. The transfection efficiency of vector/DNA nanoparticles in brain capillary endothelial cells (BCECs) was evaluated by fluorescent microscopy and determination of luciferase activity. And the potency of vector/DNA nanoparticles was evaluated by using frozen sections and measuring tissue luciferase activity in Balb/c mice after intravenous administration.

Results and discussion

The NMR results demonstrated the successful synthesis of PAMAM-PEG-Lf. It showed a concentration-dependent manner in certain concentration range in the uptake by brain capillary endothelial cells (BCECs). The brain uptake of PAMAM-PEG-Lf was 2.2-fold compared to that of PAMAM-PEG-Transferrin (Tf) in vivo. The transfection efficiency of PAMAM-PEG-Lf/DNA nanoparticle was profoundly higher than that of PAMAM-PEG-Tf/DNA nanoparticle in vitro and in vivo. The results of frozen sections showed the widespread expression of an exogenous gene in mouse brain via intravenous administration. With a PAMAM/DNA weight ratio at 10:1, the brain gene expression of the
PAMAM-PEG-Lf/DNA nanoparticle was about 2.3-fold when compared to that of the PAMAM-PEG-Tf/DNA nanoparticle. From the results described above, three possibilities for the enhanced brain accumulation of PAMAM-PEG-Lf or gene expression of the PAMAM-PEG-Lf/DNA nanoparticle can be proposed: (1) The binding between Lf and its receptors are not affected by the endogenous Lf, for the Lf receptors were not saturated under physiologic conditions because of the low plasma concentration of endogenous Lf \cite{3}, (2) Lf was positively charged itself, which can bind with the negative cellular membranes more easily than Tf, and (3) Lf transport across the BCEC monolayer was reported as unidirectional, from the apical to the basolateral side \cite{4}, which might result in higher accumulation of Lf-conjugated vector or vector/DNA nanoparticle in the neuron, compared to Tf-conjugated counterparts.

**Conclusion**

These results provide evidence that PAMAM-PEG-Lf can be exploited as a potential non-viral gene vector targeting to the brain via noninvasive administration. Lactoferrin is a promising ligand for the design of gene delivery systems targeted to the brain.

**References**

Introduction
Bivalent ligand strategy has been utilized in the design of dual binding site AChE inhibitors. Homo- or hetero-bivalent ligands are obtained by connecting two identical or distinct moieties through a linker of suitable length to contact both the catalytic and peripheral sites of AChE. In many cases of homobivalent ligands (bis-ligands), their AChE inhibitory potency and selectivity improved relative to the monomer and dual inhibition of AChE-induced Aβ aggregation was observed.

Experimental
Bis-(-)-nor-meptazinols (bis-(-)-nor-MEPs) 5 possessing methylene spacers varying from 2 to 12 were designed and synthesized. They were tested in vitro for AChE and BChE inhibitory potency. A molecular docking study was performed to illuminate the binding modes of the most potent compound 5h with both enzymes. The ability of compounds 5f, 5g, 5h, and 5i to inhibit the AChE-induced Aβ aggregation was assessed by means of a thioflavin T-based fluorometric assay.

Results and discussion
The most potent nonamethylene-tethered dimer 5h exhibited low-nanomolar IC_{50} values for both ChEs, having a 10,000-fold and 1500-fold increase in inhibition of AChE and BChE compared with (-)-MEP. Molecular docking elucidated that 5h simultaneously bound to the catalytic and peripheral sites in AChE via hydrophobic interactions with Trp86 and Trp286. In comparison, it folded in the large aliphatic cavity of BChE due to the absence of peripheral site and the enlargement of the active site. A 400 μM concentration of 5h and 5i inhibited AChE-induced Aβ aggregation by 92% and 82%.

Conclusion
Our results suggested that bis-(-)-nor-MEPs 5 are promising disease-modifying agents for the treatment of AD patients. Further pharmacological study is needed to evaluate their abilities to reverse memory impairment in animal models in order to select ideal candidates for the treatment of AD patients.
$5 \ (n=2-12)$

![Chemical structure image]

![Graph showing ChEs inhibitory potency -log(IC50) vs chain length (n) for AChE and BChE]
STRATEGY TO PREVENT MATRIX LAMINATION AT GASTRIC pH VIA FORMATION OF A CROSS-LINKED BARRIER

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Introduction

Sodium alginate matrices have been shown to sustain the release of a highly water soluble drug. However, matrices of certain alginate grades cracked or laminated during dissolution in acidic media, compromising their intended function. This was attributed to the conversion of sodium alginate to alginic acid. It has been suggested that calcium alginate gel is stronger than the corresponding alginic acid gel and therefore less susceptible to mechanical damage due to swelling pressure. In addition, it was reported that the conversion of calcium alginate to alginic acid at pH < 3 did not change the morphology or structure of alginate beads. Hence, it was postulated that alginate matrix integrity could be preserved by cross-linking, thereby enabling sustained drug release at gastric pH.

Experimental

Matrix tablets consisting of sodium alginate, chlorpheniramine maleate (model drug) and magnesium stearate (lubricant) were immersed in 0.1 or 0.01 M calcium chloride solution for 0.5, 1 or 1.5 h. The cross-linked matrices were then oven-dried at 40 ºC overnight and stored in a desiccator for a day prior to dissolution testing. Dissolution studies were carried out using USP method A (pH 1.2 for 2h, followed by pH 6.8).

Results and discussion

Preliminary studies showed that the incorporation of calcium salts into alginate matrices to bring about in situ cross-linking failed to retard drug release at gastrointestinal pH. In contrast, alginate matrices immersed in non-acidic medium containing calcium ions sustained drug release for up to 24 h due to the formation of calcium alginate barrier. Calcium alginate-coated matrices were therefore prepared and tested under simulated gastrointestinal pH conditions.

Alginate matrices pre-coated by cross-linking with calcium ions showed markedly reduced drug release rates. Drug release in the acidic phase (2 h) decreased from 60 % to 22 % or less with cross-linking. Even with gradual transformation of calcium alginate to alginic acid, matrix integrity was preserved. Matrices cross-linked in 0.1 M calcium chloride solution showed lower drug release rates in the acidic phase than matrices treated in 0.01 M calcium chloride solution. This was due to lower barrier permeability brought about by more extensive cross-linking at higher cross-linker concentration. Burst release was observed shortly after pH change which coincided with the appearance of cracks in some of the matrices.
Conclusion

Alginate matrices pre-coated with calcium alginate by external cross-linking were able to sustain the release of a freely soluble drug at pH 1.2 followed by pH 6.8 for over 12 h, mainly by preserving the integrity of the diffusion barrier.

References


Introduction

Angiogenesis is essential for the growth and persistence of solid tumors and their metastases. Folkman has defined angiogenesis as an appealing target for the development of antineoplastic drugs. Antiangiogenic therapy is recently becoming a research focus of cancer pharmacology and dozens of angiogenic inhibitor including endostatin are undergoing clinical evaluations.

Endostain, a Mr 20000 COOH-terminal fragment of collagen XVIII was initially isolated from the conditioned medium of hemangiondethelioma cells. It suppresses the growth of primary human and murine tumors and metastases in different xenograft mouse tumor models. However, the half-life of endostatin in mice after S.C. injection of 50mg/kg/day is only 10h. This would provide a peak plasma level but not a continuous level of endostatin. And continuous administration of endostatin improves the efficiency of therapy.

The purpose of this work is to design biodegradable microspheres for sustained delivery of endostatin and investigate effects of different polymer types on the endostatin release from microspheres.

Experimental

Endostatin was microencapsulated into end-group capped and uncapped poly(lactic-co-glycolic)(PLGA) by using a w/o/o multiple emulsification-evaporation technique. Microspheres were characterized for their encapsulation efficiency and release characteristics in phosphate-buffered saline(PBS) at pH 7.4 and 37°C. Moreover, the stability of the peptide during 28 days of release was evaluated using HPLC techniques. Pharmacokinetics was also evaluated in rats.

Results and discussion

Emulsification-evaporation produced microspheres in the size range of 10–70 μm, and with encapsulation efficiencies varying between 46% and 95%. In vitro release of endostatin followed a regular pattern and lasted more than 4 weeks. Microspheres made of 14-kDa end-group uncapped PLGA50:50 or 40 kDa end-group uncapped...
PLGA50:50 gave the best release profiles and yielded the most sustained plasma levels above a pre-defined 100 ng/ml over approximately 14 days. This study demonstrates that controlled release of the endostatin is possible in vivo for a duration of at least 2 weeks when administered S.C. to rats.

**Conclusion**

These results constitute a step forward towards a twice-a-month microsphere-formulation for the treatment of cancers.

**References**

IDENTIFICATION OF IN VITRO PHASE I METABOLITES OF MEISOINDIGO IN RAT AND PIG LIVER MICROSONES BY LC-MS/MS

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Introduction

Metabolism is a key determinant of the in vivo fate of a drug molecule. The metabolic transformation can lead to the generation of inactive metabolites, active metabolites, reactive intermediates or toxic metabolites. Hence, a good understanding of the properties of drug metabolites plays a critical role in the drug development. Meisoindigo has been approved for the three phases of clinical trials for the treatment of chronic myelogenous leukemia (CML) in China. However, few publications relevant to the drug metabolism of meisoindigo have been reported so far. The aim of this article is to identify in vitro phase I metabolites of meisoindigo in rat and pig liver microsomes.

Experimental

The control and experimental samples from incubation with male Sprague-Dawley (SD) rat and Large White-Landrace (LL) pig liver microsomes were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The mobile phase consisting of 10mM ammonium acetate and methanol was eluted in linear gradient mode through a reversed-phase analytical column (5 μm hypersil ODS, 100 ×2.1 mm). The mass spectrometer was operated in the positive ion mode with a TurboIonSpray source. Enhanced Q3 Single MS (EMS) and Enhanced Product Ion (EPI) were utilized to elucidate the structures of the major metabolites of meisoindigo. The metabolites were identified based on their molecular masses and fragmentation patterns. UPLC-QTof was also used to provide the accurate masses and potential formulae to further confirm the structures of metabolites.

Results and Discussions

The total ion chromatograms (TIC) of the control and experimental samples were compared. The corresponding EPI mass spectra of the major metabolites were compared with those of the parent drug. The major in vitro phase I metabolites of meisoindigo in rat microsomes were tentatively identified as reduced-meisoindigo (m/z 279), monohydroxy-reduced-meisoindigo (m/z 295) and N-demethyl-reduced-meisoindigo (m/z 265). The major metabolites in pig microsomes were tentatively identified as reduced-meisoindigo (m/z 279).
Conclusion

The tentative structures of \textit{in vitro} phase I metabolites of meisoindigo in rat and pig microsomes were proposed respectively.

References

Introduction

Poly-L-lactic (PLLA) and poly (N, N’ - (dimethylamino) ethyl methacrylate) (PDMAEMA) are extensively used in biomedical and pharmaceutical applications, especially in tissue engineering and drug delivery. However, the cytotoxicity to cells of PDMAEMA limits the further application in biomaterials. Many groups have studied the physicochemical characteristics of the surface modified biomaterials and their influences to cell behaviors, but little research has been performed on the surface gene transfection efficiency of surface-modified biomaterials. In this article, we reported the gene transfection into mammalian cells on the DMAEMA grafted PLLA film.

Experimental

The surface of PLLA film was modified with DMAEMA via UV-induced graft copolymerization. Fourier-transform infrared attenuated total reflection and X-ray photoelectron spectroscopy were performed to verify the efficiency of the UV-induced graft copolymerization. The graft density was determined with a C.I. Acid Orange 7 dyeing method for the DMAEMA grafted PLLA film. Gel filtration chromatography was used to evaluate the weight average molecular of DMAEMA polymer chains.

pSV-β-galactosidase plasmid DNA molecules were absorbed onto the surface of modified PLLA film by electrostatic interactions with cationic DMAEMA polymer. L929 mouse fibroblast was cultured on the surface of DNA adsorbed DMAEAM modified PLLA film. Optic microscopy was used to randomly take photos of the cultured cells. At the same time, the cytotoxicity of the films was evaluated with 3-(4,5-dimethylthiazd-2-yl)-2,5-diphenylentrazolium bromide assay. Transfection efficiency of each experimental group was measured for β-galactosidase activity through O-nitrophenyl-β-D-galactopyranoside assay by using UV spectrophotometer.

Results and discussion

X-ray photoelectron spectroscopy and Fourier-transform infrared attenuated total reflection spectra show that plasmids are adsorbed on the DMAEMA-modified film. From the uptake of ionic dyes, DMAEMA graft density increases with the time of UV-induced graft copolymerization. DNA adsorption assay suggests that plasmid adsorption density on DMAEMA-modified PLLA film is proportional to DMAEMA graft density. The weight average molecular of grafted DMAEMA polymer chains was estimated from
the elution time of gel filtration chromatography for homopolymers using poly (ethylene glycol) as standard.

Cell morphology observation and cytotoxicity assay suggested that the DMAEMA grafted PLLA film had obvious cytotoxicity to the cells. On the contrary, cytotoxicity of the surface was highly decreased after adsorbed with plasmid DNA. In gene transfection experiment, it is assumed that the density of DNA adsorbed on the modified PLLA film increases with the time of UV-induced graft reaction, which gives rise to more DNA molecules entering into cells and gene transfection efficiency increasing. In the tranfection system of low DNA amount, DMAEMA grafted PLLA film has a higher transfection efficiency than lipofactamine. We suggest that the enhancement of gene transfection efficiency of DMAEMA grafted PLLA film is due to the maintenance of an elevated concentration of DNA on the cultured cell surface. This result indicates that DMAEMA grafted PLLA film has a potential application in the in situ gene therapy.

Conclusion

This study shows that DMAEMA graft polymerized PLLA film is able to promote the transfection efficiency of plasmid DNA and markedly decrease the cytotoxicity. This DNA adsorbed DMAEMA modified PLLA has the ability to deliver DNA into cells with high transfection efficiency and have potential application in gene active materials and in situ gene therapy.

References

POSTER PRESENTATIONS
MACHINE LEARNING APPROACH FOR LIGAND-BASED VIRTUAL SCREENING OF LARGE COMPOUND LIBRARIES WITH IMPROVED HIT-RATE AND ENRICHMENT FACTOR

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Introduction

Virtual screening (VS) has been extensively explored for facilitating lead discovery and for identifying agents of desirable pharmacokinetic and toxicological properties. Machine learning (ML) methods have recently been used for developing ligand-based VS (LBVS) tools to complement or to combine with structure-based VS (SBVS) and other LBVS tools for improving the coverage, performance and speed of lead discovery.

ML LBVS tools tend to show lower hit-rate (fraction of known hits in the predicted hits) than the best performing structure-based VS (SBVS) tools, partly because they have been trained by limited spectrum of inactive compounds. We examined to what extent hit rate and enrichment factor of ML-LBVS tools can be improved by using training-sets of more diverse spectrum of inactive compounds. A widely used ML method, support vector machines (SVM) was used to develop SVM-LBVS tools for identifying HIV-1 protease inhibitors, dihydrofolate reductase (DHFR) inhibitors, dopamine receptor antagonists, and central nervous system (CNS) active agents.

Experimental

These SVM-LBVS tools were trained by using known active compounds and putative inactive compounds extracted from compound families that contain no known active compound. Compound families can be generated by clustering distinct compounds of chemical databases into groups of similar structural and physicochemical properties. The developed SVM-LBVS tools were tested in screening libraries of 2.986 million compounds in the PUBCHEM database that are not in the training sets of our developed SVM-LBVS tools. The percentages of the known hits predicted, hit-rates and enrichment factors derived from these tests were compared with those of SBVS and other SVM-LBVS tools applied in the screening of extremely-large libraries to determine to which extent the overall performance of SVM-LBVS can be enhanced and whether it is comparable to that of the best performing SBVS tools. To further evaluate whether these SVM-LBVS tools predict active and inactive compounds rather than membership of certain compound families, distribution of the predicted active and inactive compounds in the compound families were analyzed.
Results and discussion

The developed SVM-LBVS tools for identifying HIV protease inhibitors, DHFR inhibitors, dopamine antagonists, and CNS active agents in screening 2.986 million distinct compounds in the PUBCHEM database that are not in the training sets of our developed SVM-LBVS tools. There are 2,351, 225, 37, and 664 known HIV protease inhibitors, DHFR inhibitors, dopamine antagonists, and CNS active agents in the PUBCHEM database not in the training sets of our developed SVM-LBVS models. Our developed SVM-LBVS tools were able to identify 78.0%, 52.4%, 62.2%, and 66.6% of these known hits, which are comparable to the range of 62%–95% by the SBVS tools and 55%–81% by other SVM-LBVS tools in screening libraries of ≥1 million compounds, and they are also comparable to the percentages in screening libraries of 98,400–344,500 compounds by other SBVS and LBVS tools. These results suggest that our developed SVM-LBVS tools are equally effective in selecting potential hits in VS of large libraries.

The hit-rates of our SVM-LBVS tools are 22.5%, 73.8%, 7.7%, and 4.7% for the four classes of compounds respectively, which are comparable to those of 0.65%–35% by SBVS tools and substantially improved against those of 0.2%–0.7% by other SVM-LBVS tools in screening extremely large libraries. These hit-rates are also greater than the majority of the hit-rates in screening large libraries of 98,400–344,500 compounds by SBVS and other LBVS tools. The enrichment factors of our SVM-LBVS tools are 296, 10,543, 6,417, and 214 for the four classes of compounds respectively, which are substantially improved against those of 20–1,200 by SBVS tools and 110–795 by other SVM-LBVS tools in screening extremely large libraries. Therefore, our method is useful in improving the hit-rate and enrichment factor of SVM-LBVS tools while maintaining the equally high hit identification rate as other SBVS and LBVS tools.

Conclusion

By using training sets of more diverse spectrum of inactive compounds, the hit-rates and enrichment factors of SVM-LBVS tools can be substantially improved to the level comparable to and in some cases higher than those of the best performing SBVS tools. Because of their high computing speed and capability for covering highly diverse spectrum compounds, SVM and other ML methods can be potentially explored to develop useful VS tools to complement other VS methods or to be used as part of integrated VS tools in lead discovery.

References

SYNTHESIS OF 6-AMINO-1-METHYL-1,3,5-TRIAZINAN-2,4-DIONES

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INTRODUCTION

The 1,3,5-triazine heterocycle has been found in several bioactive molecules. Cycloguanil, an antimalarial agent contains a 4,6-diamino-1,2,-dihyro-1,3,5-triazine nucleus while the Baker’s 1,3,5-triazines have been reported to possess anticancer property. To date there are several strategies reported in the literature on the synthesis of 1,3,5-triazine derivatives. For instance, 1,3,5-triazine has been generated from 3-atom amindines, 5-atom biguanides and 6-atom urea derivatives. In addition, reports on the synthesis of 1,3,5-triazines from nitriles, isocyanates, imidates and carbodiimides are also available.

This study adopted a strategy that used ethoxycarbonyl isothiocyanate as the starting material for the preparation of the 1,3,5-triazine. In addition, in the design of the synthetic schemes, the inclusion of two keto groups in the 1,3,5-triazine nucleus was considered, mainly due to the hypothesis that the two keto groups were required for the interaction of the target enzyme, neuronal nitric oxide synthase. As such, the objectives of this study was to develop a solution phase approach and a solid phase approach to preparing a few small libraries of 6-amino-1-methyl-1,3,5-triazinan-2,4-diones.

EXPERIMENTAL

The following synthetic schemes were developed for the solution phase and solid phase synthesis of the 6-amino-1-methyl-1,3,5-triazinan-2,4-diones.
RESULTS AND DISCUSSION

The three synthetic schemes were used successfully in the preparation of fourteen 6-amino-1-methyl-1,3,5-triazinan-2,4-diones, namely 5a-c, 10a-d, 12a-d and 14a-c. Both the solution phase and solid phase syntheses proceeded well with the use of ethoxycarbonyl isothiocyanates. The target triazines were fully characterized.

CONCLUSION

This study demonstrated the feasibility of using ethoxycarbonyl isothiocyanate as the starting material in both solution and solid phase synthesis of 6-amin-1-methyl-1,3,5-triazinan-2,4-diones.
FOUR NEW PRENYLATED 2-ARYLBENZOFURANS FROM THE ROOT BARKS OF *ARTOCARPUS PETELOTII*

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**Introduction**

*Artocarpus* species (Moraceae) are evergreen plants distributed over tropical regions of Asia, such as Indonesia, Thailand, and Sri Lanka. Primarily known for their large edible fruits, some members have important medicinal value. Especially in Indonesia, many *Artocarpus* plants are used as traditional folk medicine called “Jamu” against inflammation, malarial, fever, dysentery, and tuberculosis. Previous research work on this genus provided a variety of prenylated flavonoids and some stilbenes and 2-arylbenzofurans with chemical and biological diversity. In a program searching for bioactive prenylated phenols from Chinese *Artocarpus* plants, we have investigated the chemical constituents of *Artocarpus chama* Buch.-Ham. and *Artocarpus petelotii* Gagnep, both plants being cultivated in Xishuangbanna, Yunnan province, China. It is interesting that their phenolic constituents found so far are different. The roots and stems of *A. chama* are rich in prenylated flavones with cytotoxicity, prenylated stilbenes and their biogenetic derivatives being minor constituents. On the other hand, a series of prenylated 2-arylbenzofurans, artopetelins A–G, were isolated from the root barks of *A. petelotii*. The limited distribution of 2-arylbenzofurans in *Artocarpus* genus and their interesting bioactivities, such as antibacterial, antiviral, cancer-chemopreventive, and cyclooxygenase-inhibitory effects, have attracted our attention. Therefore, a re-examination of the EtOH extract from the root barks of *A. petelotii* yielded four new prenylated 2-arylbenzofurans, artopetelins H–K (1–4). This paper describes the isolation and structure elucidation of these compounds.

**Experimental**

The air-dried and powdered root barks (6.4 kg) of *A. petelotii* were percolated with 95% EtOH (60 l) at room temperature. The filtrate was concentrated to give a residue (800 g), which was suspended in H₂O (2 l) and partitioned successively with petroleum ether (4×800 ml) and EtOAc (4×800 ml). Combined with a series of column chromatograph such as silica gel, RP-18, MCI gel CHP-20P, Sephadex LH-20 and Toyopearl HW-40C, compounds artopetelins H–K (1–4) were isolated from the EtOAc extract.

**Results and discussion**

Compounds artopetelins H–K (1–4) were discovered for the first time. They enriched the chemistry of prenylated 2-arylbenzofurans which distributed in limited *Artocarpus* genus, and furthermore provided important evidence for chemotaxonomy.
Conclusion

Four new prenylated 2-arylbenzofurans, artopetelins H–K (1–4), were isolated from the root barks of *Artocarpus petelotii* Gagnep. Their structures were elucidated by extensive 1D-, 2D-NMR, and MS spectral analysis.

References

THE ACTING TIMING OF FACTORS ON THE DRUG RELEASE FROM THE SEMIPERMEABLE COATED TABLET

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Introduction
The releases of water-soluble drug from the semipermeable coated tablets have been widely reported. However, no reports have been found about discussing the effect of the complex action of other factors on drug release. The present study is to explain the compositive effect of osmotic pressure, the solubility and release speed of excipients, the swelling of the tablets and the viscosity of solution on drug release. The effects of timing and condition of each factor on drug release were analyzed. Different excipicents were applied to prepare different inner cores with Salbutamol sulfate (SB). Hydroxypropylmethylcellulose (HPMC) and the mixture of Eudragit RS and RL were applied as the hydrophilic swelling layer and semipermeable outer coat, respectively. In vitro release test of SB and excipien ts and the swelling test of the coated tablets were investigated. The results showed that the release rate of SB from the same coating level tablets depended on the complex action of the following four factors: osmotic pressure, the release speed of excipients, the swelling of the coated tablets and the viscosity of solution formed by drug and excipient. The present study provided more detail data which could be referred to design the ideal dissolution semipermeable coated tablets.

Experimental
Hydroxypropylmethylcellulose (HPMC) and the mixture of Eudragit RS and RL were applied as the hydrophilic swelling layer and semipermeable outer coat, respectively. In vitro release test of SB and excipients and the swelling test of the coated tablets were investigated. The swelling tests of these semipermeable coated tablets were also investigated.

Results and discussion
The acting timing of osmotic pressure on drug release is that osmotic pressure only decided the time needed to form the cracks on the coated film and the size of the cracks. The cracks formed on the coated film resulted in the instantaneous release of the drug dissolved in the solution. The remanent drug which had not been dissolved was released to the medium by diffusion, while not by osmotic pumping effect. These results indicated that, for the device of bilayer semipermeable coated tablet which was depended on the cracks formed on the coated film to release drug, the release rate of drug of the tablets was not dependent on the value of osmotic pressure after the lag time.

Conclusion
After the lag time, the release rate of drug from the same coating level tablets depended on the complex action of the diffusion area, the viscosity of the solution in
the tablets and the release rate of excipients. However, the osmotic pumping was not the key factor affecting the drug release after the lag time. In a word, when we need to design the semipermeable coated tablets of ideal dissolution, we should consider the effect of complex action of all these factors on the drug release profiles.

References
COMPARATIVE STUDY OF THE ANTIPLATELET AND ANTICOAGULANT ACTIVITIES OF DIFFERENT PAXAN SPECIES

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Introduction

Panax notoginseng, a well-known Chinese medicinal herb, is available as raw and steamed forms, each with different pharmacological actions and chemical profiles. It belongs to the same family and genus as P. ginseng (Chinese ginseng) and P. quinquefolium (American ginseng). However, to date, the inhibitory activities of these species on platelet aggregation and coagulation have not been compared. Therefore, the objective of this study is to determine the effects of raw and steamed P. notoginseng, P. ginseng (Chinese white ginseng and Korean red ginseng) and P. quinquefolium (American ginseng), on platelet aggregation and blood coagulation pathways.

Experimental

Raw and steamed P. notoginseng, P. ginseng, P. quinquefolium and Korean red ginseng were extracted and studied. Platelet aggregations (in vitro) were determined using whole blood aggregometer. Blood coagulation assays (in vitro), namely, Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), were performed using a blood coagulation analyser.

Results and discussion

For platelet aggregation assays, raw and steamed P. notoginseng roots were found to inhibit platelet aggregations, and the steamed samples showed significantly greater inhibition effects than the raw samples. Compared to other widely used Panax species, P. notoginseng have the greatest inhibitory activities. In coagulation assays, raw and steamed P. notoginseng significantly prolonged PT and APTT, with the steamed samples being prolonged to a greater extent than raw samples.

Conclusion

P. notoginseng is a useful medicinal herb with antiplatelet and anticoagulant activities and its activities are higher than other related Panax species. These results also highlight the impact of steam processing on P. notoginseng and the importance of standardising this process for consistent activities.

References

STATISTICAL LEARNING APPROACHES FOR PREDICTING PHARMACOLOGICAL PROPERTIES OF PHARMACEUTICAL AGENTS

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Introduction

Drug development is aimed at the finding of therapeutic agents that possess desirable pharmacological properties, which includes pharmacokinetic, pharmacodynamic and toxicological profiles. Historically, inappropriate pharmacological properties have been the primary reasons for the failure of drug candidates in the later stages of drug development. Thus tools for predicting pharmacological properties in early drug design stages are needed for fast elimination of agents with undesirable properties so that development efforts can be focused on the most promising candidates. As part of the efforts for developing such tools, computational approaches have been explored for predicting various pharmacological properties of pharmaceutical agents.

This work aims to study the applicability of statistical learning methods (SLMs) [Li et al. 2006b] to classify compounds from diverse structures into different pharmacological properties. Specifically, the pharmacokinetic models explored in this work are activators for pregnane X receptor (PXR) [Ung et al. 2006]. The pharmacodynamic model studied in this work is agonists of estrogen receptor (ER) [Li et al. 2006a] and the toxicity model studied is genotoxicity (GT) [Li et al. 2005] agents.

Results and Discussions

A set of 199 molecular descriptors is used to describe the molecular physicochemical properties of those pharmaceutical agents studied in this work. A feature selection method recursive feature elimination (RFE) is incorporated to improve the prediction performance. The results show that SLMs can improve the quality of these pharmacological properties prediction models by using enlarged and more diverse groups of compounds. RFE is able to identify a group of relevant molecular descriptors that reflect the pharmacological properties of studied models and are consistent to quantitative structure activity relationship (QSAR), pharmacophore and X-ray crystallographic studies. In addition, selection of appropriate molecular descriptors can lead to substantially more balanced prediction accuracies and enhance the overall accuracies. Moreover, SLMs are found to be useful for developing prediction models and characterizing relevant physicochemical features for PXR activators and ER agonists, which are very important pharmacological properties of drug candidates but insufficiently explored in the previous studies.
Conclusion

Our studies show that SLMs, particularly SVM, are useful for facilitating the prediction of pharmacological properties of a diverse set of molecules, such as the PXR activators, the ER agonists and the genotoxic agents, without requiring the intrinsic mechanism knowledge of chemical compounds. SLMs consistently show promising capability for predicting chemical agents of diverse ranges of structures and of a wide variety of pharmacodynamic, pharmacokinetic, and toxicological properties. Thus, it is likely that SLMs, especially SVM, are efficient computational tools for the prediction of pharmacological properties of pharmaceutical agents.

References


POLYOXYPOLYETHYLENE 40 STEARATE MODULATES MULTIPLE DRUG RESISTANCE AND ENHANCES ANTITUMOR ACTIVITY OF VINBLASTINE SULPHATE

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Introduction
Multiple drug resistance (MDR) is known to endure over expression of P-glycoprotein (P-gp) [1]. Hence, strategies to inhibit P-gp in vivo have become an attractive approach regarding novel cancer therapy. Some surfactants used in pharmaceutical formulation, including polyoxyethylene 40 stearate (PS40), have been found to be able to enhance the absorption of P-gp substrates in vitro [2]. However, the effects of PS40 on cancer therapy are still unknown.

In the present study, vinblastine sulphate (VBS) was used as the test drug to define PS40’s effects on cancer therapy. Transport experiments through Caco-2 cell monolayers and rat intestine tissue were done to find out whether PS40 can modulate MDR in vitro. Adriamycin-resistant human chronic myelogenous leukemia (K562/ADR) cells were used in the drug efficacy experiment both in vitro and in vivo for their over expression of P-gp. MTT cytotoxicity assay and multi-drug resistant tumor-bearing nude mice experiment were done to define whether PS40 can enhance cancer therapy effects of VBS.

Experimental
1. Transport experiments in Caco-2 monolayers and rat intestine tissue
Caco-2 cell monolayers were used when Transendothelial electrical resistance (TEER) exceeded 300Ω×cm². The transport studies were performed similar to that described in previous studies[2,3]. Donor solutions of 200μg/ml VBS without or with different concentrations of PS40 in TBS were added to the donor compartment. The final concentration of PS40 was 50, 100 and 150μg/ml, respectively.

The transport experiments performed in rat intestine tissue were similar to that described in the previous study [4]. Donor solutions of 200μg/ml VBS without or with different concentrations of PS40 in TBS were added to the donor compartment. The final concentration of PS40 was 50, 100 and 150μg/ml, respectively.

2. MTT assay
The effects of PS40 on VBS’s cytotoxicity to K562/ADR cells were determined in quadruplicate using MTT cell viability assay as previously described [5]. PS40 was added into the test VBS solution. Then cytotoxicity of VBS to K562/ADR cells was assessed. The final concentrations of PS40 were 0, 50, 100 and 150μg/ml while the final concentrations of VBS were 50, 100, 120, 180, 200 and 220μg/ml. After 8 hours treatment, cells were incubated for 4 hours in the presence of MTT reagent and then lysed with DMSO. Absorbance was measured at A490 nm. Each experiment was repeated four times or more (n≥4).

3. tumor-bearing nude mice
Female BALB/c-nu/nu mice were age-matched (4 weeks of age) at the beginning of each experiment. The K562/ADR cells were inoculated subcutaneously into the left side of the armpit of athymic nude mice (1×10^7 cells/each). The mice were assigned randomly to three groups (n = 4) after inoculation. After tumors were formed to 2.0mm×2.0mm, mice in the control group was treated with 0.9% sodium chloride solution. Vinblastine sulphate was injected subcutaneously at a dosage of 2 mg/kg VBS around the tumor every other day for 8 days (VBS group). VBS and PS40 were injected subcutaneously at a dosage of 2 mg/kg VBS and 1.5mg/ kg PS40 around the tumor every other day for 8 days (VBS+PS40 group). The volume of tumors and the weight of nude mice were measured everyday since the day tumors were formed.

**Results and discussion**

In Caco-2 monolayers, PS40 reduced the BL-AP versus AP-BL ratio (i.e. B/A ratio) of VBS transport. In the absence of PS40, the B/A ratio was 10. With 50, 100 and 150 μg/ml PS40, the respective ratio was 9, 8 and 4.

In rat intestine, PS40 reduced the BL-AP versus AP-BL ratio (i.e. B/A ratio) of VBS transport. In the absence of PS40, the B/A ratio was 1.80, with 50, 100 and 150 μg/ml PS40, the respective ratio was 1.50, 0.88 and 0.58.

K562/ADR cell survival rates were significantly lower with PS40 at 100 and 150 μg/ml.

There was no significant difference in the mean volume and mean weight of tumor between control group and VBS group. The mean volume and mean weight of tumor in VBS +PS40 group were significantly lower.

**Conclusion**

PS40 can modulate MDR in vitro and enhance the drug’s therapy effect. PS40 may be used in cancer therapy.

**References**


CHARACTERIZATION OF FUNCTIONALIZED SWNT AND A PRELIMINARY STUDY ON THEIR IMPACT ON IMMUNE CELLS

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Introduction
Carbon nanotubes have shown the potential as drug delivery systems. In this study we functionalized the single wall carbon nanotube (SWNT) by conjugating it with primary amino groups and investigated the cytotoxicity and immune response of functionalized SWNT (f-CNT).

Experimental
1. Cutting of SWNT
   About 20 mg SWNT was suspended in H2SO4-HNO3 (3:1) and sonicated to obtain cut and oxidized SWNT. After neutralizing the acids, the suspension was filtered through a 0.2 µm membrane. The SWNT cake on the filter membrane was washed using distilled water.

2. Functionalizing of SWNT
   A suspension of oxidized tubes in oxalyl chloride was stirred at 62 °C for 24 h. Boc-NH(CH2CH2O)2-CH2CH2NH2 was added and refluxed for 48 h. The suspension was filtered through 0.2 µm membrane and washed with methanol for several times to remove the un-reacted material. After that, so obtained SWNT was suspended in 4 M HCl in dioxane solution and stirred for 5 h to cleave the Boc group. The resulted functionalized SWNT (f-CNT) was washed with diethyl ether for several times and dried under vacuum. The extent of functionalizing was measured with the quantitative Kaiser test.

3. Cytotoxicity and immune response of f-CNT
   Cytotoxicity was assessed by culture B- and T-cells in the presence of f-CNT. The cell viability was measured by flow cytometry. Stimulatory effect of f-CNTs on lymphocytes was tested depending on the proliferation of B- and T-cells while the activation of macrophage was monitored by the level of TNFα and IL6.

Results and discussion
1. Cutting of SWNT
   Oxidized SWNT, with higher solubility, was obtained after sonication for 6 h. Dispersion of SWNT in water (1 mg/ml) might keep stable for at least one week. The increased hydrophilicly was caused by –COOH groups introduced during the oxidation.

2. Functionalizing of SWNT
Quantitative Kaiser test showed that the loading amount of the prime amino group was 0.11 mmol per gram of f-CNT. The solubility of the f-CNT was about 2 ~ 3 µg/ml in water.

3. Cytotoxicity and immune response of f-CNT
   No any significant loss of cell viability upon incubation of the three cell types was observed. In addition, the f-CNT induced neither higher proliferation of B- and T-cell nor activation of macrophages.

Conclusion
Our results showed that the SWNT could be oxidized and cut by H2SO3-HNO3. The resulted SWNT showed increased solubility. Moreover the introduced –COOH groups render it possible for further chemical modification on the SWNT.

Prime amino groups were conjugated on the SWNT via an oxalyl chloride mediated reaction. The prime amino groups may entitle the f-CNT positive charge and at same time retained a solubility of 2 ~ 3 µg/ml in water.

Biological experiments indicated that the f-CNT is neither cytotoxic nor immunogenic, suggesting f-CNT’s promising potential as carriers of biological and therapeutic molecules.
SUPERIORITY OF HYPERICIN DELIVERY BY N-METHYL PYRROLIDONE OVER ALBUMIN FOR CANCER DETECTION IN THE CHICK CHORIOALLANTOIC MEMBRANE MODEL

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Introduction
Fluorescence diagnosis of cancer using photosensitizers is one of the major advances as a new technique for early cancer detection.¹ Clinical application of hypericin (HY) in photodynamic diagnosis (PDD) has demonstrated very high sensitivity and specificity to detect human bladder cancer significantly better than white light endoscopy.² ³ However, its lipophilicity complicates the administration of HY for clinical applications. Conventionally, plasma protein is used as an ‘effective’ HY transporter/carrier. An alternative acceptable pharmaceutical preparation for HY would be desirable. Currently pharmaceutical preparations without plasma protein are being developed.⁴ Formulations containing a biocompatible solvent, N-methyl pyrrolidone (NMP) was shown to enhance the photodynamic therapy (PDT) of HY.⁵ ⁶ This present work hypothesizes that NMP acts as a penetration solvent enhancer and is able to improve the delivery of HY into cancer cells implanted in the Chick Chorioallantoic Membrane (CAM) model for better fluorescence diagnosis of cancers.⁷

Experimental
The safety of NMP was evaluated in human bladder carcinoma MGH cells and CAM. Preparation of the CAM for experimentation was carried out in the tissue culture hood to avoid problems relating to contamination. Fertilized eggs were first disinfected with 70 % alcohol before placing them in the egg incubator at 37 °C with 60 % humidity. At embryo age (EA) of 6 – 7 days, a window was opened at the blunt end facing upwards using sterilized forceps. The opening was then covered with parafilm to avoid contamination and the egg returned to the incubator. On the next day, 6-12 X 10⁶ MGH cells were transplanted on each CAM. The window was again sealed and the eggs were further incubated till EA 12 – 13. The conventional formulation of HY (HY-HSA 0.5 %) used in the clinic was included as a control. The red-to-blue (I(R)/I(B)) intensity ratio of fluorescence images was used as a diagnostic algorithm, to differentiate the uptake of HY between tumor and adjacent regions on CAM.

Results and discussion
Survival data showed that MGH cells were able to tolerate NMP concentrations up to 1 %. Concentration of NMP lower or equal to 4.8 % did not damage CAM. The tumor selectivity of HY was not compromised when it is formulated with NMP. HY-NMP 0.05 % was able to produce a significantly higher contrast between the tumor and its adjacent tissue in the CAM, at 5 h earlier than the conventional HY-HSA 0.5 % formulation. An interval, 30 min after application of the test formulation showed that
HY-NMP 0.05 % treated CAM had a four- to five-folds higher HY fluorescence intensity compared to CAM treated with HY-HSA 0.5 %.

The fluorescence enhancement effects by HY-NMP formulations could be due both to the presence of NMP and/or absence of albumin. CAM tissue may not mimic the true physiological conditions of normal bladder urothelium. However, the direct application of HY formulations on the CAM implanted with the MGH cells simulates the clinical bladder instillation of HY preparation. When HY is currently used for PDD of bladder cancer, HY is prepared as a bladder instillation fluid using human plasma protein. The reported disadvantage of this preparation was that the instilled solution must be in contact with every part of the bladder for as long as 2 h. This administration will not be suitable for patients with intravesical blood clots, diverticula, small bladder capacities or lacking mobility. The findings of the $I(R)/I(B)$ in tumor and adjacent tissues supported the potential usefulness of NMP as the alternative to human plasma protein, in clinical PDD using HY. With improved HY-NMP formulation for more efficient delivery of HY to the tumor cells, the rate of uptake of HY can be enhanced. A good contrast between the tumor and normal regions at earlier time point post-drug administration is obtained. This will not only allow a low drug dose to be used but will also significantly reduce patient’s waiting time during treatment.

**Conclusion**
The HY-NMP formulations investigated were able to produce significantly higher contrast for tumor cells and at earlier time points than was possible with the conventional albumin-based formulation of HY.

**References**
FERMENTATION EFFICIENCY OF GELLAN GUM MICRO-BIOREACTORS
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Introduction
There is strong commercial interest in using immobilised microbial cells as bioreactors for fuel production from biomass because of the increasing demand in renewable energy sources to supplement the depleting fossil fuels. The use of encapsulated microbial cells as bioreactors for ethanol production has been reported to offer advantages such as higher productivity and lower production cost. Microbial cells immobilised in polymeric beads or microspheres to form bioreactors for fermentation to produce ethanol were found to be protected from the toxicity of their own metabolites, especially ethanol. The viability of the immobilised cells is thus preserved and this enables higher fermentation efficiency of the bioreactor. Natural polysaccharides such as gellan gum have been used to encapsulate microbial cells for various applications. Gellan gum is a linear anionic polymer composed of repeating units of tetrasaccharide of β-D-glucose, β-D-glucuronic acid and α-L-rhamnose residues. Gellan gum is potentially useful for encapsulation of microbial cells as bioreactors because of its stability towards high temperature. Besides, it also forms acid and enzyme resistant gels unlike other polysaccharides commonly used in cell encapsulation such as alginites.

Experimental
Encapsulation of Yeast Cells
One g of yeast containing 1 X 10^9 cells was dispersed into 50 g of sterilised gellan gum solution. The mixture was then dispersed in 75 g of iso-octane containing 2.2 g of Span 80 by using a mechanical stirrer for 10 min. 5 g of aqueous solution containing 1.7 g of Tween 80 was then added and the mixture was stirred for another 5 min. The temperature of the mixture was kept at 40 °C from the beginning of the experiment. The mixture was then cooled to 15 °C with the aid of an ice water bath. Further stirring was continued for 15 min to induce gelation of the dispersed droplets of gellan gum solution. 20 g of 25 %w/w calcium chloride solution was then added and allowed to react for 15 min. The reaction mixture was finally left to stand in a shaker water bath for 2 h to enable complete congealation of the gellan gum. The microspheres formed were harvested by filtration in vacuo and rinsed with 10 ml of sterile de-ionised water and then washed again with 100 ml of sterile de-ionised water to remove the iso-octane residue completely.

Fermentation
The microspheres with encapsulated yeast cells were inoculated into 300 ml of malt extract broth containing 30 %w/w sucrose which was then shaken in a shaker water bath at 90 strokes per min under anaerobic condition at 30 °C. Samples were taken at different time intervals for assay of ethanol. Fermentation using the same number of free yeast cells to replace the encapsulated cells was used as the control for this experiment.

Assay of Ethanol
Ethanol was recovered from the fermentation medium by the solvent extraction method and assayed by GC-MS with a BP-Wax column (0.25µm film thickness, 30 m X 0.25 mm I.D.) and acetone as internal standard. Each sample was assayed in triplicate.
Results and Discussion

Yeast cells were successfully encapsulated in gellan gum microspheres (Figure 1). Presence of free yeast cells was not detected in the freshly harvested samples of microspheres by microscopic examination.

The free yeast cells were found to achieve maximum ethanol production of 16.27% after 5 days of fermentation (Figure 2). A drop in ethanol yield was observed after a week of fermentation, possibly due to oxidation of the ethanol to ethanoic acid or loss of ethanol by evaporation. It was also possible that the cells were adversely affected by prolonged exposure to the toxic effects of ethanol. Reduction in cell viability due to nutrient deficiency or toxic effects of cumulative metabolites is well-established.

The yeast cells encapsulated in gellan gum microspheres achieved the maximum ethanol production of 13.36% only after 12 days of fermentation (Figure 2). Ethanol production by encapsulated yeast cells in gellan gum was lower and took a longer time. This phenomenon might be caused by the stress induced on the yeasts by the encapsulation process or by impairment of mass transfer by the polymer matrix. The reduction rate of ethanol production was found to be lower for encapsulated yeast cells (average of 3.52% per day) compared to that for free yeast cells (5.50% per day). This shows that the gellan gum matrix provided a protective function to the encapsulated yeast cells. The gellan gum microspheres were still intact and were not disintegrated at the end of the fermentation study that spanned 17 days, indicating that the matrix is strong. However, free yeast cells were observed in the fermentation medium. The encapsulated cells were able to detach from the microspheres indicating that the matrix was porous (Figure 1c).

Conclusions

Yeast cells were successfully encapsulated in gellan gum microspheres using the emulsification method. The yeast cells were not significantly harmed by the encapsulation process and were able to perform fermentation. However, the ethanol production was lower and the micro-bioreactors took a longer duration to reach its maximum ethanol yield. Gellan gum is a very stable polymer and suitable for cell encapsulation, however, further experiments have to be conducted to improve the matrix porosity of the microspheres to overcome the cell leakage problem.
USE OF MACHINE LEARNING METHODS IN THE STUDY OF TRADITIONAL CHINESE MEDICINE FROM TRADITIONAL POINT OF VIEWS

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Introduction

Traditional Chinese medicine (TCM) has been widely practiced and is considered as an attractive alternative to conventional medicine. Multi-herb recipes have routinely been used in TCM. These have been formulated by using TCM-defined herbal properties (TCM-HPs) where the scientific basis is unclear. In addition, these multi-herb prescriptions often include special herb-pairs for mutual enhancement, assistance, and restraint. These TCM herb-pairs have been assembled and interpreted based on TCM-HPs without knowledge of mechanism of their assumed synergy. While these mechanisms are yet to be determined, properties of TCM herb-pairs can be investigated to determine if they exhibit features consistent with their claimed unique synergistic combinations.

Experimental

Machine learning methods (MLMs) such as support vector machine (SVM), k-nearest neighbors (kNN), and probabilistic neural network (PNN) are used to analyze the distribution pattern of TCM-HPs of the constituent herbs in 1,161 classical TCM prescriptions and 394 TCM herb-pairs.

Results and discussion

MLMs correctly classified 83.1%~97.3% of the TCM prescriptions, 90.8%~92.3% of the non-TCM recipes. In addition, distribution patterns of TCM-HPs of TCM herb-pairs were analyzed using MLMs to detect signs indicative of possible synergism. Patterns of the majority of 394 known TCM herb-pairs were found to exhibit signs of herb-pair correlation. By using 394 TCM herb-pairs and 2,470 non-TCM herb-pairs, MLMs correctly classified 72.1%~87.9% of TCM herb-pairs and 91.6%~97.6% of the non-TCM herb-pairs. The best MLM system predicted 96.3% of the 27 known non-TCM herb-pairs and 99.7% of the other 1,065,100 possible herb-pairs as non-TCM herb-pairs.
Conclusion

Our studies suggested that TCM-HPs of known TCM multi-herb prescriptions and herb-pairs contain features distinguishable from those of non-TCM recipes and herb-pairs consistent with their claimed synergistic or modulating combinations.

References


A COMPARATIVE STUDY OF AIR VELOCITY DISTRIBUTION IN THE WURSTER AND PRECISION COATERS

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Introduction

Coating has found important applications in the pharmaceutical industry, including protecting the active drug from the environment, taste masking, improving appearance, enhancing mechanical strength, and modifying drug release behaviour[1]. Wurster and precision coating are two bottom spray fluidized bed processes for pellet coating. The two coaters differ in their air distribution modes. The precision coater employs a swirl accelerator to generate swirl air while the Wurster coater does not have the swirl accelerator. It has been reported that the precision coater is able to yield better pellet coating quality than the Wurster coater[2]. Although this has been attributed to their different air flow patterns during coating, little effort has been put in to date to carefully examine the air flow patterns in these two coaters. Hence, this study aimed to investigate and visualize the air velocity distribution patterns in the partition columns of the Wurster and precision coaters.

Experimental

Wurster and precision coating were conducted on the respective coaters using the same air handling system (MP-1, GEA-Aeromatic Fielder, UK). Partition gap was set at 10 mm. Air flow rate was increased from 80 m³/h to 120 m³/h with a step of 10m³/h while atomizing air pressure was increased from 0 bar to 3.0 bar with a step of 0.5 bar. The air velocity in the partition column was measured using a Pitot tube (160-12,Dwyer Instruments, U.S.A.) placed at four different positions, 0 mm, 0.5 mm, 1.5 mm, and 2.5 mm away from the center of the partition column. Twenty readings were taken at each experimental condition and averaged. Matlab (Version 7.0.1.24704 (R14), The MathWorks, U.S.A.)was then employed for visualization of the air velocity distribution patterns.

Results and discussion

The overall air velocity in the precision coater was found to be higher. In general, the change in air velocity with air flow rate and atomizing air pressure at all four positions in the partition column was also observed to be greater in the precision coater than in the Wurster coater. While air velocity in the Wurster coater was mainly dependent on atomizing air pressure, air velocity in the precision coater was influenced by both air flow rate and atomizing air pressure.
Conclusion

The higher overall air velocity in the precision coater could generate higher drag force and high pellet velocity, thus minimizing agglomeration during coating. The additional air flow rate dependent property of the precision coater enabled more robust yet flexible air flow patterns to be generated in its partition column and a greater range of coating conditions for use in pellet coating.

References


IRIDOID GLYCOSIDES FROM *HEDYOTIS CORYMBOSA*

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Introduction

*Hedyotis corymbosa* (Linn.) LAM. is an annual herb widely distributed in the southeast and southwest of China. The whole plant is applied in clinic against malaria, intestinal abscess, boils, scald, and some kinds of tumors, such as gastric, esophageal, and colorectal carcinomas. Some iridoid glycosides were isolated from this plant previously, but the bio-activities of these compounds were not investigated. In this paper, we describe the structural elucidation of three new iridoid glycosides.

Experimental

The whole plant of *H. corymbosa* (Linn.) LAM. was bought from Shanghai Medical Material Corporation, in July 2003, and air-dried. The plant was identified by Prof. Sheng-Li Pan, Fudan University, and a voucher specimen (TCM 03-07-03 Hou) was deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University. The compounds were isolated by means of column chromatography, prep. TLC and so on. The structures of the new compounds were elucidated by extensive 1D- and 2D-NMR analysis as well as by HR-ESI-MS experiments.

Results and Discussion

Our phytochemical research on the n-BuOH fraction from an ethanol extract of the whole plant of *H. corymbosa* afforded three new iridoid glycosides, hedycorysides A–C (1–3), along with four known compounds, 10-O-benzoyl scandoside methyl ester (4), 10-O-\(p\)-trans-coumaroyl scandoside methyl ester (5), 10-O-\(p\)-hydroxybenzoyl scandoside methyl ester (6), and 10-O-benzoyl deacetyl asperulosidic acid methyl ester (7).

Conclusions

In *Hedyotis* genus, most of iridoid glycosides contain the skeletons of scandoside, deacetyl asperulosidic acid, and deacetyl asperuloside. Although geniposide derivatives are quite common in iridoid glycosides, only geniposidic acid has been found in *Hedyotis* plants. To our knowledge, hedycorysides A–C (1–3) are the first three benzoylated geniposide derivatives from *Hedyotis* species.
OPTIMIZATION OF COATING FORMULATION FOR YEAST-LOADED ALGINATE MICROSPHERES EMPLOYED FOR BIO-FUEL PRODUCTION

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Introduction
Immobilization of microorganisms as bioreactors for fermentation to produce bio-ethanol has attracted great interest in the search for renewable energy to replace the depleting fossil fuel supplies. Encapsulation of cells in microspheres allows the use of high cell load besides protecting the cells from toxic substances, thereby enabling higher ethanol yield. Alginate is commonly used as a polymer matrix for microencapsulation of microorganisms. However, due to its porous polymer matrix, breakthrough of cells often occurs. Coating of the microspheres with an appropriate formula was hypothesized to overcome the breakthrough of cells without retarding mass transfer through the coat. Hence, the aim of this study was to investigate the suitability of various coating formulations of a water-insoluble polymer, ethylcellulose (EC).

Experimental

Microencapsulation of Yeast
Microspheres containing yeast were prepared by the emulsification method. One hundred g of 8 %w/w sodium alginate (Sigma, USA) with a known amount of yeast cells (Turbo Extra Yeast, Still Spirit, New Zealand) were dispersed in 150 g of isooctane (Merck, Germany) containing 5.65 g of Span 85 (Sigma, USA) and stirred at 1000 rpm for 10 min. Ten g of aqueous solution containing 7.35 g of Tween 85 (Merck, Germany) were then added and stirring continued for another 5 min. Fifty g of 25 %w/w calcium chloride (Merck, Germany) solution were finally added and stirred for 10 min. Microspheres were harvested by filtration in vacuo after standing for 2 h. These microspheres were then dispersed in sterile water and the suspension was passed through a 600 μm mesh. The microspheres in the filtrate were then collected by filtration in vacuo and subjected to coating by a spray-drying method.

Coating of microspheres
The freshly prepared microspheres were added into various aqueous ethylcellulose dispersions (EC, Aquacoat® ECD-30, FMC Corporation, USA) with 30 %w/w triethyl citrate (TEC, Merck, Germany) and different concentrations of trehalose (Trehal®, Hayashibara, Japan), based on the weight of the polymer. The dispersion was stirred for 5 h with a magnetic stirrer. Weight ratio of microspheres to polymeric dispersion was varied at 2:1 and 1:1 ratio. The mixture was then spray-dried (Mobile Minor, Niro, Denmark) to coat the microspheres with the polymer at a rotary atomiser speed of 23000 rpm and inlet and outlet air temperature of 140 °C and 60 °C respectively.

Fermentation efficiency
Coated alginate microspheres containing yeast cells were subjected to fermentation in 250 g of fermentation medium containing 30 %w/w sucrose (SIS, Singapore) and 5 g of malt extract (Oxoid, UK). Ethanol produced was recovered by the solvent extraction method with 2-ethyl-1-hexanol (Merck, Germany) and analysed by gas chromatography (HP 5890 Series II, USA) equipped with a DB Wax column (1 μm film thickness, 30 m x 0.53mm I.D., J&W Scientific, USA) and flame ionization detector. Each sample was assayed in triplicate.
Results and discussion
The ethanol yield for the various coating formulations is shown in Figure 1. Ethanol production was insignificant for the alginate microspheres coated at 1:1 ratio without trehalose. The amount of ethanol produced by those coated at 2:1 ratio, without or with 25% trehalose, increased gradually with fermentation time, giving 13.37% at day 7 and 12.32% at day 6 respectively. In contrast, the ethanol yield for those coated at 2:1 ratio with 10% trehalose increased from day 4 to day 5 to a peak at 13.84% and then decreased gradually with time. The amounts of ethanol produced for microspheres coated at 2:1 ratio, with or without trehalose were comparable, ranging form 12% to 14%, but the time taken to reach maximum ethanol production was markedly longer for the coating without trehalose. Coating at ratio of 1:1 was not suitable as excessive aggregates of coating material were found with the coated microspheres (Figure 2a). Microscopic examination showed that the amount of free yeast was minimal in all the samples before fermentation. However, after fermentation, more free yeast cells were observed for coating formulation with higher trehalose concentration. Trehalose created pores in the coat when it dissolved in the fermentation medium. While the pores facilitated mass transfer, they enabled breakthrough of cells. A balance between these two effects is important for efficient fermentation. Among the different coating formulations studied, coating at 2:1 ratio with 10% trehalose was found to be the most appropriate.

![Figure 1. Ethanol concentration produced by yeast-loaded alginate microspheres coated by different formulations. (□) 1:1 ratio without trehalose, (■) 2:1 ratio without trehalose, (♦) 2:1 ratio with 10% trehalose and (▲) 2:1 ratio with 25% trehalose.](image)

![Figure 2. Photographs of yeast-loaded alginate microspheres coated by different formulations (a) 1:1 ratio without trehalose, (b) 2:1 ratio without trehalose, (c) 2:1 ratio with trehalose](image)

Conclusion
Yeast-loaded alginate microspheres were successfully coated by the spray drying method. Appropriately coated yeast-loaded microspheres were able to produce significant amount of ethanol by fermentation. The fermentation efficiency was affected by the porosity of the coat around the microspheres. The breakthrough of cells and the mass transfer through the coat can be controlled by the addition of hydrophilic additives.
A DNA VACCINE AGAINST TAENIA SOLIUM CYSTICERCOSIS EXPRESSED AS A MODIFIED HEPATITIS B VIRUS CORE PARTICLE CONTAINING THREE EPITOPES SHARED BY TAENIA CRASSICEPS AND TAENIA SOLIUM

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Introduction

*Taenia solium* cysticercosis is a common parasitic disease of humans in several countries in Latin America, Africa, and Asia, where it represents a major health and economic problem. Several approaches are being used currently toward the development of a *T. solium* vaccine, including vaccines using native and recombinant oncosphere antigens from whole *T. solium* cysticercus, 3–7 protein subunit vaccines, and DNA vaccines. In research of vaccines against parasites, the protective antigen is the most important factor. Many studies have provided evidence that the hepatitis B core antigen particle is useful as a vaccine carrier for foreign epitopes. Epitopes KETc1, KETc12, and GK-1 are three promising candidates for designing a vaccine against *Taenia solium* cysticercosis. Based on the knowledge above, we constructed an expressed plasmid encoding a truncated HBc protein (amino acids 1–149) with two epitopes (KETc1 and KETc12) inserted between HBc amino acids 78 and 79, a region located at the tip of the core particle surface spikes, and one epitope (GK-1) fused to its C-terminus. Afterward, we constructed a DNA vaccine pVAX-ΔC-3n and studied the immune protection against *T. solium* in pigs.

Experimental, Results and discussion

In the present study, epitopes KETc1 and KETc12 were inserted into the immunodominant loop of the truncated HBc149, and epitope GK-1 was fused to its C-terminus. The fused protein ΔC-3n was expressed and purified successfully. The polymeric character was tested by SDS-PAGE. After inoculation of BALB/c mice with ΔC-3n, antibody titers were assayed by ELISA, and the antibody specification was analyzed by Western blot. Dot ELISA was performed to verify the protection of the three epitopes. Results showed that the purified polymeric protein was formed, high antibody titers were induced in immunized mice and three antibodies different in molecular weight were induced, serum specific antibody recognized the native peptide localized mainly in cyst wall cells, and there was no specific antibody toward the three epitopes in sera of infected pig and humans. All these revealed that the protein ΔC-3n was a potential candidate for vaccine against cysticercosis. So the ΔC-3n sequence and the signal peptide sequence of IL-2 were cloned to a vector pVAX3.0 to construct pVAX-S-ΔC-3n. Pigs were immunized with pVAX-S-ΔC-3n. Two weeks after the immunization booster, pigs were introduced to infectious *T. solium* eggs.
Conclusion

pVAX-ΔC-3n could protect the immunized pig well from the challenge of *T. solium* eggs.

References

THE EFFECT OF SALVIANOLIC ACID B AND LAMINAR SHEAR STRESS IN THE ATTENUATION OF TNF-A-INDUCED ICAM-1 EXPRESSION IN HUMAN AORTIC ENDOTHELIAL CELLS

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Introduction
Intracellular adhesion molecule-1 (ICAM-1) is one of inflammatory adhesion molecules as biomarkers of atherosclerosis\textsuperscript{1}. Laminar shear stress (LSS) is an essential regulator of inflammatory response on endothelial cells in the occurrence of atherosclerosis. Exposure of endothelial cells to LSS has been shown to atheroprotective\textsuperscript{2}. Salvianolic acid B (Sal B), an active compound of the \textit{Salvia miltiorrhiza} Bunge (Danshen), an eminent herb for cardiovascular diseases, can attenuate the expression of ICAM-1 in TNF-\textalpha treated human aortic endothelial cells (HAECs)\textsuperscript{3}. The purpose of the present study was to further investigate the combined protective effects of Sal B and LSS on the expression of ICAM-1 by TNF-\textalpha- treated HAECs.

Experimental
Inhibitory effect of Sal B on the TNF-\textalpha (2 ng/ml for 4 h) induced ICAM-1 expression in HAECs was assessed at different concentrations (10, 25 and 50 µg/ml) for 24 h. The parallel plate flow chamber has been developed to control the experiments for LSS (12 dynes/cm\textsuperscript{2} for 24 h) on cultured endothelial cells\textsuperscript{2}. Both flow cytometry and western blotting were employed to detect ICAM-1 expression. Data were analyzed by ANOVA followed by the LSD test (\(p<0.05\)).

Results
It was found that Sal B could significantly attenuate ICAM-1 expression in a dose-dependent manner (\(p<0.05\)). The expression of ICAM-1 was also decreased in the HAECs by the pre-treatment of LSS. Interestingly, Sal B enhanced the attenuating effect of LSS on TNF-\textalpha-induced ICAM-1 expression in HAECs.

Conclusions
Sal B has a potent effect on the inhibition of ICAM-1 expression. Both LSS and Sal B showed combined protective effect on TNF-\textalpha-induced ICAM-1 expression in HAECs.

References
FLOATING MATRIX DOSAGE FORM FOR PHENOPORLAMINE HYDROCHLORIDE BASED ON GAS FORMING AGENT: IN VITRO AND IN VIVO EVALUATION IN HEALTHY VOLUNTEERS

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Introduction
Phenoporlamine hydrochloride (DDPH) is a novel compound that is used for the treatment of hypertension. The purpose of this study was to develop a sustained release tablet for DDPH because of its short biological half-life. Three floating matrix formulations of DDPH based on gas forming agent were prepared. Hydroxypropyl methylcellulose K4M and Carbopol 971P NF were used in formulating the hydrogel drug delivery system. Incorporation sodium bicarbonate into matrix resulted in the tablet floating over simulated gastric fluid for more than 6 h. The dissolution profiles of all tablets showed non-Fickian diffusion in simulated gastric fluid. Moreover, release of the drug from these tablets was pH-dependent.

Experimental
The tablet formulations evaluated consisted of DDPH, sodium bicarbonate, polymer(s), fillers and magnesium stearate. Three different DDPH tablets were formulated to produce different drug release profiles. These formulations contained 15% Carbopol 971P, 7.3% HPMC K4M (formulation A), 25% Carbopol 971P, 5.3% HPMC K4M (formulation B) and 25% Carbopol 971P, 8.3% HPMC K4M (formulation C). Each formulation contained 60 mg of DDPH, 75 mg sodium bicarbonate and 1.5% (w/w) of magnesium stearate. The total weight of the entire tablet was maintained around 300 mg.

The in vitro release rates of DDPH from the matrix tablets were determined using the USP XXVI (basket apparatus). The basket rotation speed was kept at 150 rpm, and a temperature of 37±0.5 °C was maintained. Release testing was carried out in 900 ml of different dissolution media: pH 1.2 artificial gastric fluid, pH 6.8 phosphate buffer and distilled water. Five-milliliter samples were withdrawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h. The samples were replaced by its equivalent volume of dissolution medium and were filtered through a 0.8µm filter and assayed at 278 nm by UV spectrophotometry. Six tablets of each formulation were used in the dissolution test, over the range of 10–100 µg/ml. High accuracy and precision.

Male healthy volunteers in a group of six participated the in vivo study. The mean age of the volunteers was 22 years (range 20–26 years), mean weight 65 kg (range 62–70 kg), and mean height 168.2 cm (range 164–175 cm). Three open, single dose, randomized crossover trials with at least four weeks intervals were conducted to evaluated concentrations in plasma after administration of three formulations and the reference product. Each subject received a 60 mg dose as one tablet. Each treatment period in every trial was followed by a one-week wash out period to eliminate the effects of the tested dose before the next treatment. Blood samples were collected over 24 h after each drug
administration. Plasma samples were assayed for DDPH concentration using the HPLC procedure.

All results are expressed as mean±S.D. Differences between two related parameters were considered statistically significant for p-values of or less than 0.05. Analysis of variance (ANOVA) was carried out on the pharmacokinetic parameters. Bioequivalence between the test and reference formulations was assessed by a two one-sided test procedure via 90% confidence intervals from analysis of relative BV values.

Results and discussion

The release of DDPH from three different formulations was determined in different media. Anon-Fickian diffusion was confirmed as the drug release mechanism from these tablets. This meant that water diffusion and the polymer rearrangement have essential roles in the drug release. In order to prolong the gastric residence time, a gas forming agent was used to keep the tablets floating over simulated gastric fluid for more than 6 h. The in vivo experiments showed that keeping the amount of Carbopol 971P NF at a higher concentration effectively increased the relative bioavailability of drug. In addition, a good correlation between in vivo and in vitro drug release was obtained in the formulation B and C. The statistical bioequivalency was achieved for the relative bioavailability of formulation C with an acceptable range of 98–123%.

Conclusion

The gastric floating matrix tablets prepared from mixtures of Carbopol 971P and HPMC K4M proved to be useful devices for DDPH oral delivery systems.

References

ITS SEQUENCE ANALYSIS USED FOR MOLECULAR IDENTIFICATION OF THE BUPLEURUM SPECIES FROM NORTHWESTERN CHINA

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Introduction
Radix Bupleuri (Chaihu), a famous Traditional Chinese Medicine, is derived from the dried roots of \textit{Bupleurum chinense} DC. and \textit{B. scorzonerifolium} Willd.. But due to the slight different morphologic appearance of \textit{Bupleurum} plants and their dried roots, there were 36 \textit{Bupleurum} species and varieties under the same name of Radix Bupleuri in different districts and markets, including \textit{B. longiradiatum} Turcz. with toxic ingredients and \textit{B. hamiltonii} Balak with very little active constituents (Pan, 1996; Pan \textit{et al}., 2002). In northwestern China, there are approximately 11 species utilized nowadays. Inevitably, this situation will compromise the values of Radix Bupleuri and even imperil the safety of the consumers. Traditional means of Radix Bupleuri (Chaihu) authentication rely on the inspection of morphological markers such as shape, color, texture and odor. Its accuracy depends heavily on the examiner’s experience. Therefore, more objective and definitive methods are necessary. Herein, the ITS region of nuclear ribosomal DNA was employed to assist identification, for its valuable source of phylogenetic information, particularly at the infragenetic and infrafamilial level (Baldwin, 1992, 1993; Baldwin \textit{et al}., 1995). It’s the first time to identify all the \textit{Bupleurum} species used in northwestern China by ITS sequence analysis.

Experimental
Total cellular DNA was extracted from the ground powder of 20 populations representing 11 species by using a modified CTAB method (Doyle and Doyle, 1987). DNA amplifications were performed in 25 \textmu l reaction mix. Amplify primers were ‘ITS 5P’ (Möller and Cronk, 1997; the same as ‘modified ITS 5’ in Downie and Katz-Downie, 1996) and ‘ITS 4’ (White \textit{et al}., 1990). The PCR profile consisted of an initial 5 minutes at 93°C, 2 minutes at 55°C then following by 30s at 93°C, 45s at 55°C, 45s at 70°C, repeated for 35 cycles and with 5 minutes extension at 70°C. Clustal X, 1.8, was used for multiple alignment of complete sequences. Pairwise distances between taxa and base frequencies (G+C content) were determined using MEGA 3.1. Phylogenetic analyses were performed using PAUP\textsuperscript{*}, 4.0b10. Maximum likelihood (ML) analysis was performed by quartet puzzling using TREE-PUZZLE 5.0. Distance tree was obtained by neighbor-joining analyses, using two of the distance measures available in MEGA 3.1: Jukes and Cantor and Kimura 2-parameter models.

Results and discussion
20 ITS sequences were successfully amplified and sequenced. ITS1, with a maximum of 50.4% divergence across all taxa (20.1% in \textit{Bupleurum}), is slightly shorter and more variable in length than ITS2, which has a maximum of 54.2% divergence across all taxa (14.2% in \textit{Bupleurum}). The 5.8S is 163 bp long and shown little variations as expected. The overall of ITS divergence was approx. 14% within \textit{Bupleurum}. Parsimony analysis of 614 characters resulted in 14 retained most-parsimonious (MP)
trees, each 292 steps long, consistency index (CI) 0.89 and retention index (RI) of 0.83. In all the MP, ML and NJ analyses, Bupleurum appears as a strongly supported monophyletic group. The division of the genus into two major clades is also consistent among all trees. The main difference between the MP, ML and NJ trees is the position of clade E (B. chinense clade). This clade appears as the first branch in the MP tree, but as sister clade to clade D (Sinkiang species) in NJ tree and in the ML tree; therefore, the relationship within clades was not so certain that it needs more samples and more analyses to demonstrate much clearer.

Conclusion
1. By comparing all the ITS sequences studied in this paper, it turned out that almost every species had their own specific variable bases, therefore ITS sequence could serve confidently as informative traits of diagnosing these local-used Chaihu. The availability of their genetic sequences could also lead to the development of gene chip application in their identification. 2. It is the first time that species from Sinkiang area have been studied in genetic analysis, although there are many Bupleurum species having been investigated (Neves and Watson, 2005). Therefore their phylogenetic relationship within the whole genus Bupleurum does merit the further study. 3. We suggested that the species which have close genetic properties and phylogenetic relationships with official species, could serve as new sources of the original plants of Radix Buplueri, after chemical analysis on the main effective ingredients—saikosaponins and volatile oils etc.

References
DEVELOPMENT OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY WITH MICROWAVE DISTILLATION AND SIMULTANEOUS SOLID-PHASE MICROEXTRACTION FOR RAPID DETERMINATION OF VOLATILE CONSTITUENTS IN GINGER

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Introduction

Ginger (\textit{Zingiber officinale}) has a long history used as a spice and as a medicinal plant. Various methods can be used for the isolation and extraction of essential oil from plant materials including TCMs, which mainly included solvent extraction \cite{1}, supercritical fluid extraction (SFE) \cite{2,3}, liquid-phase microwave-assisted processes (MAP) extraction \cite{4}. However, these methods always lead to the losses of some volatile compounds, low extraction efficiency, toxic solvent residue and time-consuming. Headspace solid-phase microextraction (HS-SPME) is a relatively new sampling and concentration technique for the extraction of plant essential oils \cite{5-9}. However, conventional HS-SPME still required about 30 min for the extraction of essential oil compounds in TCMs. More recently, microwave distillation with concurrent solid-phase microextraction (MD-SPME) was first introduced for the successful isolation and concentration of essential oil components from \textit{Artemisia Selengensis} Turcz \cite{10}. MD-SPME technique combines the advantages of MAP and SPME, so, it has high extraction efficiency, need of no organic solvent, small amount of sample, and short extraction time.

In this work, MD-SPME was developed for the analysis of volatile compounds in the TCM, fresh ginger.

Experimental

The three parameters of microwave power, SPME fiber coating and irradiation time were studied in the work. The five fibers of PDMS, PDMS/DVB, CW/DVB, CAR/PDMS and PA were tested with the same microwave parameters: power of 400 W and irradiation time of 2.0 min. Then, microwave power (200, 400 and 700 W) and irradiation time (1, 2, 4, 6 min) were investigated. The optimal parameters of PDMS/DVB fiber, microwave power of 400 W and irradiation time of 2 min were used for MD-SPME of the essential oil in fresh ginger (1.0 g). The analytes extracted on the fiber were desorbed at GC injector (250 °C for 3 min), and then analyzed by GC-MS.

To demonstrate the method’s feasibility, extraction of essential oil in fresh ginger was performed using the conventional HS-SPME with the conditions of (PDMS/DVB) fiber, and extraction time of 30 min. The analytes on the fiber were determined by GC-MS. The method precision was studied.

Volatile compound analyses were carried out on a HP 6890 GC system, coupled with an HP MD5973 quadrupole mass spectrometer. The compounds were separated on an HP-5MS capillary column (30 m×0.25 mm i.d. ×0.25 μm film). Splitless injection was
employed for both conventional HS-SPME and MD-SPME samples. Compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) mass spectral library and retention indices.

**Results and discussion**

Based on the experimental results, the optimal MD-SPME conditions are: PDMS/DVB fiber, microwave power of 400 W and irradiation time of 2 min.

Fifty-four components in the essential oil were identified. They mainly included geranial (5.25%), zingiberene (15.48%), β-sesquiphellandrene (5.54%) and β-phellandrene (22.84%).

More components were isolated and extracted from ginger by MD-SPME than those by conventional HS-SPME. The proposed MD-SPME-GC-MS method identified the larger number of volatile compounds in ginger. The other fifteen components in ginger can’t be identified by conventional SPME-GC-MS. It has been demonstrated that microwave can much improve the extraction efficiencies of plant essential oil compounds.

Moreover, rapidity is another important feature of the proposed MD-SPME extraction method. On the other hand, MD-SPME required a little sample amount, no organic solvent and water in determination of essential oil compounds in ginger.

Compared with conventional HS-SPME, MD-SPME is a simple, rapid, solvent-free and efficient method for the extraction of volatile components in ginger. The developed method for the identification of essential oils in ginger has good precision.

**Conclusion**

In the work, an MD-SPME-GC-MS technique was successfully performed for the determination of volatile compounds in ginger. Fifty-four compounds were identified in ginger using the proposed method. Compared with conventional HS-SPME method, MD-SPME-GC-MS is a simple, rapid, solvent-free and efficient method for the analysis of essential oils in ginger and other fresh plant tissues.

**References**

Introduction
9-nitrocamptothecin (9-NC) is a novel, water-insoluble analogue of camptothecin which showed high potency against lung cancer with low toxic effects in vitro. However, phase II studies concluded that oral administration of 9-NC appeared to be inactive in advanced lung cancer patients. To improve the therapeutic index of 9-NC against lung cancer, pulmonary delivery stands out among other alternative administration routes because of the direct targeting of drugs to the lungs and reducing unwanted systemic activity or toxicity. For pulmonary delivery, liposomes present many advantages over other vehicles owing to relatively low toxicity and prolonged pulmonary residence time. In this study, in vitro release, in vivo tissue distribution and the damage to the lungs of 9-NC liposomes were investigated.

Experimental
9-NC liposomes were prepared by thin-film hydration method and then freeze-dried with sucrose as cryo-protectant. The liposomes were rehydrated with distilled water before use. Drug entrapment efficiency of the liposomes was determined by centrifugation method. XRD experiments were carried out to characterize the physical state of 9-NC in liposomes. In vitro release of 9-NC from liposomes was carried out in phosphate buffer saline solution (PBS, pH 7.4) by dialysis method. The tissue distribution of 9-NC liposomes and 9-NC solution was determined after pulmonary delivery to mice. The tissue distribution of 9-NC liposomes after intravenous administration was also studied. The changes of pulmonary edema index and histology of lungs in rats were investigated to evaluate the severity of the damage after pulmonary delivery of 9-NC liposomes.

Results and discussion
The entrapment efficiency of 9-NC-containing liposomes was 94.32±0.73% before freeze-drying and 91.32±0.61% after redissolved in water. The crystalline nature of the drug may be changed during the process of preparation and the results of the XRD experiment demonstrated that the drug was encapsulated into the liposomes and may be amorphous in liposomes. 9-NC was continuously released from the liposomes in PBS pH 7.4 for 24 h at 37°C, while 91.5% of 9-NC in the stock solution was released in 1h. After pulmonary delivery, the mean residence time (MRT) of 9-NC liposomes in the lungs was 3.4 times as long as that of 9-NC solution and the total AUC0-t of all tissues in mice of the liposomes was 2.2-fold higher than that of the solution, indicating that the liposomes had sustained-release characteristics. Following intravenous administration and pulmonary delivery, the targeting efficiency (Te) to the lung of 9-NC liposomes was 0.14 and 2.02, respectively, which showed that intratracheal instillation can deliver the drug mainly to the lung and decrease the accumulation of the drug in other tissues at different concentrations. The pulmonary edema index and the histological changes of the lungs in 9-NC liposome group were
significantly different from those in 9-NC solution group. The lung damage by liposomes was less severe than that by solution.

**Conclusion**

Pulmonary delivery of 9-NC liposomes could directly deliver the drug to the lung and make the drug accumulate in the lung with sustained-release characteristics, changing the disposition behavior in vivo, decreasing the toxic and side effects on other tissues and reduce the severity of damage to lungs following intratracheal instillation. The studies can provide experimental foundation for further research on 9-NC liposomal powder for inhalation.

**References**

Introduction

*Ixeris sonchifolia* (*I. sonchifolia*) is a small perennial herb (~ 0.4m tall) commonly found in dry places. It has been used as a folk medicine in invigorating circulation of blood, normalizing menstruation and eliminating blood stasis to relieve pain. Like many medicinal plants, *I. sonchifolia* has been shown to possess anticancer bioactivity. In this study, we subjected *I. sonchifolia* to extraction, isolation, and purification experiments and obtained eight highly purified compounds. Among them, two are sesquiterpene lactone gulcosides. Since most sesquiterpenes are known to possess cytotoxic effects [1], we carried out *in vitro* cytotoxic studies to determine if these two compounds are cytotoxic and thereby might play important role in the anticancer activity of *I. sonchifolia*.

Experimental

*I. sonchifolia* (5 kg) was crushed and extracted using 95% ethanol three times at 60°C. The extracts were then evaporated to dryness under reduced pressure to yield a dried residue. The dried crude extract (420 g) was then dissolved in 800 ml of water and subjected to further extraction using equal volume of either hexane or chloroform or ethyl acetate. In all, four extracts were obtained: namely Fr. 1 (hexane), Fr. 2 (chloroform), Fr. 3 (ethyl acetate) and Fr. 4 (water). Fr. 3 was subjected to further isolation by repeated column chromatography on sephadex LH-20, silica gel as well as polyamide until pure compounds were obtained. The structures of the pure compounds were elucidated by spectrum of $^1$H NMR, $^{13}$C NMR and ESI-MS.

To determine the cytotoxicity of the two sesquiterpene lactone gulcosides, different concentration of compound 1 and compound 2 were added to MCF 10A (human non-tumorigenic breast epithelial cell line) and MCF7 cells ((human tumorigenic breast epithelial cell line) and incubated for 48h. The cells were then harvested for viability assay and apoptosis evaluation using MTT assay and flow cytometric analysis, respectively.

Results and discussion

Two new sesquiterpene lactone gulcosides and six previously characterized compounds were purified from *I. sonchifolia*. The sesquiterpene lactone gulcoside structures were determined by spectroscopic methods to be 1(10),3,11(13)-guaiatriene-12,6-olide-2-one-3-O-glucopyranoside (comp.1) and 1(10),3,11(13)-guaiatriene-12,6-olide-2-one-3-O-[6’-(p-hydroxyphenylacetyl)]-glucopyranoside (comp.1).
Mammary carcinoma cell viability was decreased after 48 h exposure of compound 2 at concentrations 50-200μM. MCF 10a and MCF7 mammary carcinoma cells showed less sensitivity to compound 2 when compared to previous reports using lung carcinoma cell lines (Table 1). In contrast, there was no significant cytotoxicity observed after 48 h exposure to compound 1.

Table 1. MTT Analysis for Cytotoxic Activity Against Normal and Tumor Cell Lines in vitro (IC50, μM)

<table>
<thead>
<tr>
<th>Comp.</th>
<th>MCF10a</th>
<th>MCF7</th>
<th>A375</th>
<th>L929</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Decreased cell viability also correlated with an increase in the sub G1 fraction of propidium iodide(PI)- stained MCF7 breast carcinoma cells after treatment with 60μM compound 1. In addition to compound 2, compound 1 at 60μM also slightly increased the sub G1 fraction (Fig. 1). The vehicle-treated control group had 0.9% cells at Sub G1 as compared to 5% and 10% for compounds 1 and 2, respectively. These results suggest that compounds 1 and 2 induce a slight increase in the apoptosis of breast carcinoma cells.

Fig.1 Flow cytometric analysis of MCF7 breast carcinoma cells after treatment with compound 1 and compound 2.

**Conclusion**

Two new sesquiterpene lactone glucosides were isolated from *I. sonchifolia*. Compound 2 showed greater cytotoxicity and increased apoptosis against breast carcinoma cells when compared to compound 1. Based on previous reports, our results suggest that different cell lines may have variable sensitivity to these compounds.

**References**


STRUCTURE-BASED 3D-QSAR STUDIES ON HETEROARYLPIPERAZINE DERIVATIVES AS 5-HT3 RECEPTOR ANTAGONISTS

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Introduction
Nausea and vomiting are major side effects associated with chemotherapy, radiotherapy, and operation. Specific 5-HT3 receptor antagonists block nausea and vomiting probably by competitive inhibition at the 5-HT3 receptor sites centrally and peripherally. To date the three-dimensional (3D) structure of 5-HT3 receptors have not been elucidated yet. When the 3D structure of a receptor is available, ligand-based and structure-based drug design methods can be combined together. Structure-based 3D-QSAR method is the result of such a combination, which could provide more information for lead optimization.

Experimental
The total set of heteroarylpiperazines from reference 7 was selected to perform 3D-QSAR studies (Figure 1). The 35 compounds were randomly divided into training set (28 molecules) and test set (7 molecules) in the ratio of 4:1. All molecular modeling and statistical analyses were performed on an R14000 SGI Fuel workstation using SYBYL v6.9 molecular modeling software package. Then, the compounds were initially docked into the binding pocket of the homology model of 5-HT3 receptor using GOLD program. The docked conformations with the highest score were then extracted and used to build the 3D-QSAR models.

Results and discussion
The docked conformations of molecules in the training set were shown in Figure 2. The superposition showed that the ligands fit the binding pocket consisting of critical residues. Table 1 shows the statistical details of CoMFA and CoMSIA. The cross-validated value, $r_{cv}^2$, was 0.716 and 0.762 for CoMFA and CoMSIA, respectively. Additional 7 molecules were used to further validate the models, giving satisfactory predictive $r^2$ values of 0.582 and 0.804 for CoMFA and CoMSIA, respectively. The CoMFA and CoMSIA contour plots were also fitted into the 3D structural model of the receptor to identify the key interactions between them, which might be helpful for designing new potent 5-HT3 receptor antagonists.
Table 1. Summary of results from the CoMFA and CoMSIA analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>$r^2_{cv}$</th>
<th>N</th>
<th>$r^2$</th>
<th>SEE</th>
<th>F</th>
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</thead>
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<tr>
<td>CoMFA</td>
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<td>6</td>
<td>0.992</td>
<td>0.073</td>
<td>433.222</td>
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<tr>
<td>CoMSIA (S+E)</td>
<td>0.789</td>
<td>6</td>
<td>0.979</td>
<td>0.118</td>
<td>162.425</td>
</tr>
<tr>
<td>CoMSIA (S+E+H)</td>
<td>0.783</td>
<td>6</td>
<td>0.984</td>
<td>0.104</td>
<td>213.510</td>
</tr>
<tr>
<td>CoMSIA (S+E+A)</td>
<td>0.735</td>
<td>6</td>
<td>0.972</td>
<td>0.138</td>
<td>119.469</td>
</tr>
<tr>
<td>CoMSIA (S+E+D)</td>
<td>0.789</td>
<td>6</td>
<td>0.979</td>
<td>0.118</td>
<td>162.425</td>
</tr>
<tr>
<td>CoMSIA (S+E+H+D)</td>
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<td>6</td>
<td>0.984</td>
<td>0.104</td>
<td>213.510</td>
</tr>
<tr>
<td>CoMSIA (S+E+H+A)</td>
<td>0.762</td>
<td>6</td>
<td>0.982</td>
<td>0.109</td>
<td>191.578</td>
</tr>
<tr>
<td>CoMSIA(S+E+D+A)</td>
<td>0.735</td>
<td>6</td>
<td>0.972</td>
<td>0.138</td>
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<td>6</td>
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</table>

Conclusion: In this study, CoMFA and CoMSIA 3D-QSAR analyses were performed on the docked conformations of 28 heteroarylpiperazines as 5-HT$_3$ receptor antagonists. Both models showed good prediction capabilities in terms of $r^2_{cv}$ and $r^2$ values, while CoMFA model showed better predictive ability [SEE (Standard error of estimate) = 0.073)] than CoMSIA one (SEE=0.109). The good correlation between experimental and predicted bioactivities for 7 compounds in testing set further verified the reliability of the constructed QSAR models. The original intention of designing the heteroarylpiperazines was to analyze the activity influence of compounds with different hydrophobic group [7]. In this study, we found some implications could be drawn to improve the activity and selectivity of heteroarylpiperazines as 5-HT$_3$ receptor antagonists. For example, moderately bulky hydrophobic electropositive group substituent at the C-4 position and the introduction of some proper alkyl substituents to the C-5’, 6’ position of piperazine might be preferable to produce higher activity. Therefore, the results would be very helpful for further structural modification of heteroparylpiperazines.

References:
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