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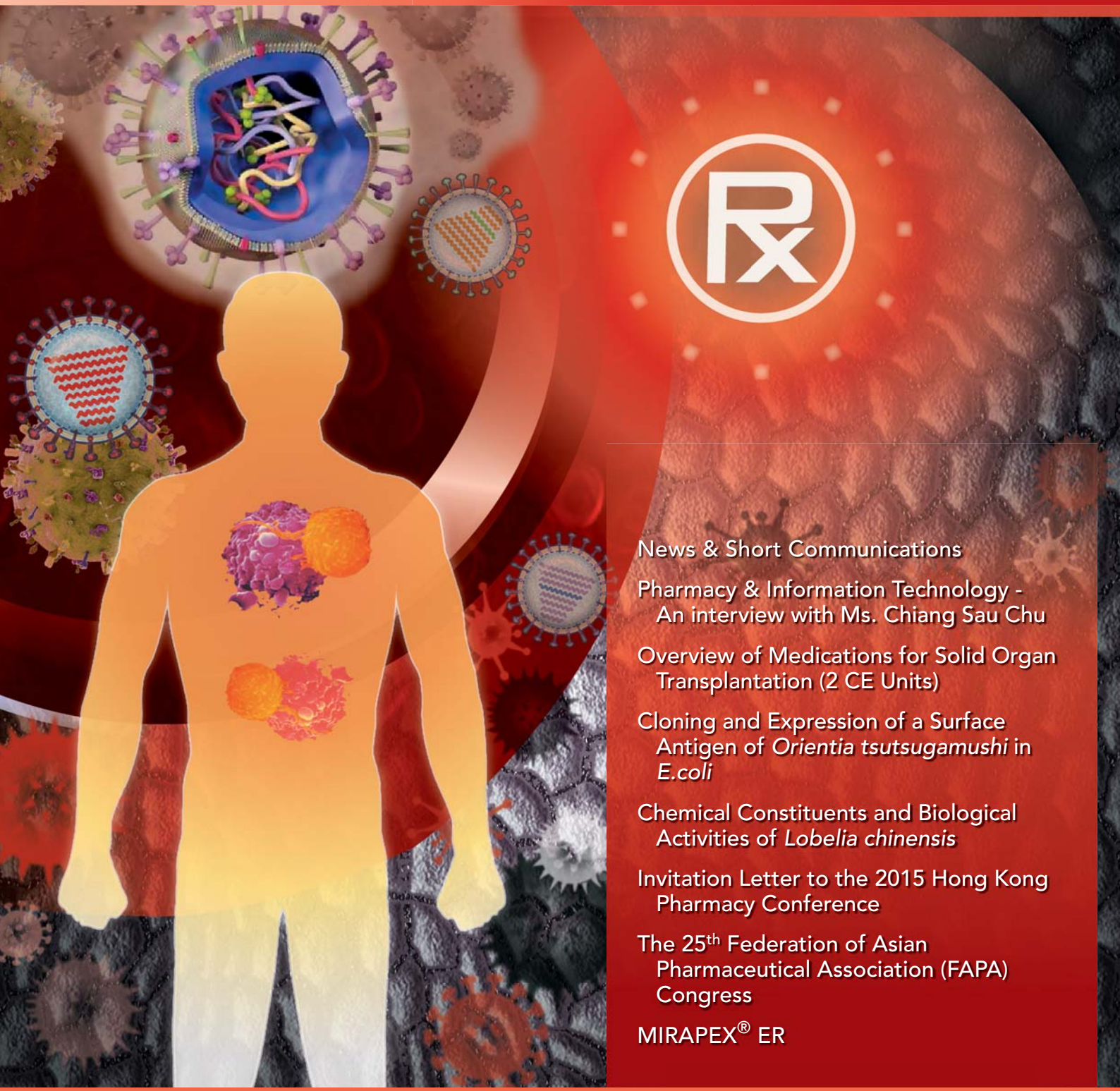

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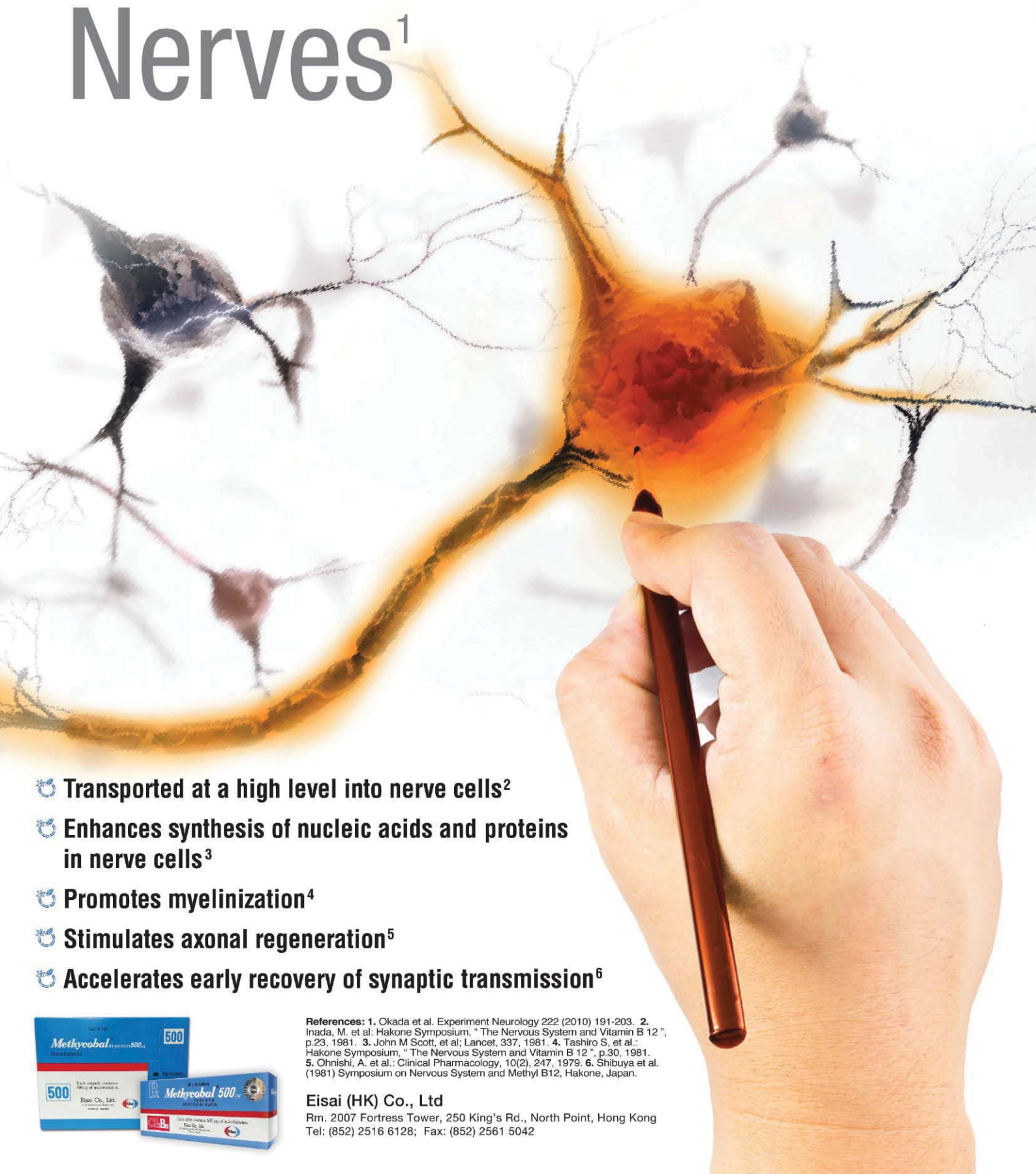

- **Transported at a high level into nerve cells[2]**
- **Enhances synthesis of nucleic acids and proteins in nerve cells[3]**
- **Promotes myelinization[4]**
- **Stimulates axonal regeneration[5]**
- **Accelerates early recovery of synaptic transmission[6]**

References: 1. Okada et al. Experiment Neurology 222 (2010) 191-203. **2.** Inada, M. et al: Hakone Symposium, " The Nervous System and Vitamin B 12 ", p.23, 1981. **3.** John M Scott, et al; Lancet, 337, 1981. **4.** Tashiro S, et al.: Hakone Symposium, " The Nervous System and Vitamin B 12 ", p.30, 1981. **5.** Ohnishi, A. et al.: Clinical Pharmacology, 10(2), 247, 1979. **6.** Shibuya et al. (1981) Symposium on Nervous System and Methyl B12, Hakone, Japan.

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INSTRUCTIONS FOR AUTHORS

The Hong Kong Pharmaceutical Journal is a journal of the pharmacists, for the pharmacists and by the pharmacists. Submissions are welcome for the following sections:

- Pharmacy Education & Practice
- Drugs & Therapeutics
- OTC & Health
- Pharmaceutical Techniques & Technology
- Medication Safety
- Herbal Medicines & Nutraceuticals
- Society Activities
- New Products

Comments on any aspects of the profession are also welcome as Letter to the Editor.

There is no restriction on the length of the articles to be submitted. They can be written in English or Chinese. The Editorial Committee may make editorial changes to the articles but major amendments will be communicated with the authors prior to publishing.

It is preferable to have original articles submitted as an electronic file, in Microsoft Word, typed in Arial 9pt. Files can be sent to the following address:

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For detail instructions for authors, please refer to the first issue of each volume of HKPJ.

The Wind that Blows out Candles Kindles the Fire

All pharmacists know that medicine is an important component in any healthcare systems. It helps to cure or attenuate disease, to relieve symptoms or just simply to convince the patient that his health problem has been dealt with even if the disease and symptoms persist. When a patient consults a medical doctor, he naturally expects at the end of the consultation to be given some medicines for the treatment of his ailment. Otherwise, he would feel disappointed if he is not given any. Equally important is the fact that medicines give veracity to the healthcare system and enhance health care facility abetment. Whatever efforts to promote health and prevent disease, for example, through immunization, behavioral change and health education in general are more likely to be acceptable in situations where patients have access to drugs than those in which medicines are totally unavailable.

However, medicines have to be properly prescribed and used with close monitoring. Unlike foods, they should not be arbitrary used without professional control or supervision. Medicines, like many other dangerous tools, could be useful but also possibly generate some undesirable effects or fatal consequences if not properly used. In the News & Short Communications section of this issue, many updated recommendations for the use of medicines endorsed by different health authorities reveal that some side effects have been discovered recently even amongst some popular and well-known drugs. To name a few instances, cautious use of Ventolin (salbutamol) in the treatment of premature labour is required; treatment of severe osteoporosis with Protoes in postmenopausal women is restricted; use of bromocriptine for stopping breast milk production is discouraged, etc and new regulations have been implemented in various countries. As a result of these new discoveries, more emphasis on drug safety and pharmacovigilance has been incorporated in contemporary pharmacy education in recent years; while in real practices, polypharmacy has been adopted in order to achieve maximal therapeutic effect and to minimize toxicity of drugs. In this issue, Wong and Mak reviewed how this tactics has been successfully applied to the recovery of a patient after solid organ transplantation.(1) In fact, Chinese medicinal practitioners are aware of the advantage of polypharmacy and have been prescribing herbal medicines to patient in compound forms for thousands of year in order to maximize the therapeutic effects and minimize the undesirable effects of substances. About two decades ago, David Ho reported that polypharmacy gave a better result for the treatment of AIDS and he cloned his practice as “cocktail therapy”.(2) A report written by Wang at el on Lobelia Chinensis Herba, which is one of the most commonly used Chinese herbs, is another example of polypharmacy from nature. This herb contains many bioactive components and polyacetylenes,(3) attributing to its anti-viral and anti-cancer functions. To learn more about the advantages of polypharmacy, readers are encouraged to read these articles.

In view of many new discoveries, pharmacists’ role today is to educate people how to use drugs in addition to traditional practices, such as drug manufacturing, dispensing and controller of drug distribution. In this innovative era, many novel and new biopharmaceuticals have been introduced. However, pharmacists do not have adequate trainings in basic biomedical sciences while handling biopharmaceuticals is almost inevitable in their daily practice nowadays. Before they are competent enough to be an expert of these medicinal substances, trainings in biotechnology should be enforced. In this issue, Cheung’s research group report how this new technology could be applied for the production of a recombinant subunit vaccine against a life threatening infectious disease, scrub typhus, due to the transmission of rickettsia in the “tsutsugamushi Triangle” of Asia.(4) The launch of thousands and thousands of biopharmaceuticals in the near future should not be neglected by pharmacists and they should be equipped with more knowledge in this area.

In the last couple of months, I have difficulties to concentrate my mind on editing this latest issue of the Hong Kong Pharmaceutical Journal. This explains why its’ publication is deferred. This delay is not simply because I have a tight working schedule and an alleged court case against me by a business man, which gives me extra-burdens and workloads, the volatile political situation in Hong Kong due to the request for true universal suffrage for the city’s leader in 2017 is really annoying. On top of this, the severe battle amongst countries in Middle East, the shaky economy in Europe and in North America, and the wide spread of Ebola virus, which is a devastating epidemic pathogen in some West Africa countries, also make me unease and worry about the future of human beings. It seems to me that the nature of human beings has not been changed even though we are living in this so-called civilized world. We are still as inconsiderate, selfish, greedy, arrogant, brutal and hypocritical as before. We don’t know how to share, to be kind, respectful, generous, loving and humble with each other. A minority of privileged people simply care for their own interest, such as power, wealth and their own way of thinking. They use their influences, muscle, network and twisting skills to manipulate the majority under the law but totally ignore the need of changes or modifications based on social justice, fairness, love and moral.

There is no law suitable forever. Law should be regularly reviewed, tuned or modified depending on situation and the needs. Furthermore, no matter how good law is, conflicts won’t disappear simply by implementing a law because dissatisfaction could be temporary suppressed but hate and distrust are retained inside the heart. Hence, it is necessary to have courage to face the social problem through sincere dialogue instead of simply asking the opponent to abide to the law. What happen if a law is bias, injustices, immoral, outdated or even twisted, don’t we have the right to ask for change. If disputes between all parties involved cannot be solved immediately, at least some schedules for further dialogues should be given. Hence, suppressive, arrogant and hypocrite attitude are not the right medication for social problems. It is remained true that the wind that blows out candles kindles the fire and I believe that the principle of polypharmacy approach or holistic approach for maintaining healthy life may be applicable to keep our society stable and prosperous too.

Cheung Hon-Yeung
Editor-in-Chief
30th November, 2014

References

1. Wong KCV, Mak VMR (2014). Overview of medication for solid organ transplantation. HK Pharm. J., 21:93-97.
2. US Food and Drug Administration (1999). Attacking AIDS with a “cocktail” therapy: Drug combo sends deaths plummeting. FDA Consumer Magazine, July-August issue, p1-8.
3. Wang HX, Zhao CY, Li YY, Huang YQ, Cheung HY (2014). Chemical constituents and biological activities of Lobelia chinensis. HK Pharm. J., 21:108-113.
4. Wong WL, Leung FM, ALQOUQA I, Cheung HY (2014). Cloning and Expression of a Surface Antigen of *Orientia tsutsugamushi* in *E. coli*. HK Pharm. J., 21:98-106.

Singapore: Transdermal Durogesic (Fentanyl): Reminder on the Potential for Life-threatening Harm from Accidental Exposure to Transdermal Fentanyl

Date: July 4, 2014

Janssen, a division of Johnson & Johnson Pte Ltd, would like to alert healthcare professionals to the potential risk of accidental exposure to transdermal Durogesic patches. The issue of accidental exposure is not a new safety issue. However, cases of accidental exposure to transdermal Durogesic in non-patch wearers, leading to fatal outcomes especially in children, continue to be reported overseas.

Healthcare professionals are reminded to provide clear information to patients and caregivers regarding risk of accidental patch transfer, accidental ingestion of patches by children, and need for appropriate disposal of used patches.

Source: www.hsa.gov.sg

Singapore: Limitations on Use of Ventolin (Salbutamol Sulphate) for Inhibition of Premature Labour

Date: July 4, 2014

GlaxoSmithKline would like to inform healthcare professionals of important restrictions regarding the use of oral and parenteral Ventolin in the treatment of premature labour. Obstetric use of short-acting beta-agonists (SABA), including salbutamol sulphate, is associated with serious or even fatal adverse cardiovascular events in both the mother and the foetus or new-born. Oral salbutamol sulphate should not be used in the treatment of premature labour and the use of parenteral salbutamol sulphate should be limited to inhibition of premature labour between the 22nd and 37th weeks of gestation for a maximum of 48 hours and administered with close monitoring of both mother and foetus. In addition, parenteral salbutamol sulphate should not be used in women with a history of heart disease or in any conditions where prolongation of the pregnancy is hazardous to the mother and foetus.

In Hong Kong, the package insert for oral Ventolin has been updated with the removal of the obstetric indication while the local package insert for parenteral Ventolin is in the process of being strengthened to include additional recommendations for screening and monitoring during management of premature labour.

However, the above revisions to the prescribing information for both oral and parenteral administration of Ventolin do not alter the prescribing information regarding the use of Ventolin for respiratory indications, including pregnant women.

Source: www.drugoffice.gov.hk

Singapore: Restricted Indication and Monitoring Recommendations for Protos (Strontium Ranelate) Following Benefit-risk Assessment by the European Medicine Agency (EMA)

Date: July 16, 2014

Servier would like to inform healthcare professionals of the restricted indication and monitoring recommendations in the local package insert of Protos to mitigate the risk of cardiovascular disorders, including myocardial infarction, following the benefit-risk assessment of strontium ranelate by the European Medicine Agency. The use of Protos is now restricted to the treatment of severe osteoporosis in postmenopausal women at high risk of fracture, for whom treatment with other medicinal products for osteoporosis is not possible due to, for example, contraindications or intolerance. Prescribers are also advised to evaluate patients with respect to cardiovascular risk before starting treatment, monitor patients' cardiovascular risk every 6 -12 months and stop treatment if the patient develops ischaemic heart disease, peripheral arterial disease, cerebrovascular disease or if hypertension is uncontrolled.

In Hong Kong, there is one registered pharmaceutical product containing strontium ranelate, namely Protos Granules For Oral Suspension 2g (HK-53835) which is a prescription-only medicine. So far, the Department of Health has not received any adverse drug reaction report on the drug. The Registration Committee of the Pharmacy and Poisons Board decided that the registered package insert of Protos should be updated to include the appropriate safety information. The DH will remain vigilant on any safety updates related to the drugs and actions taken by overseas regulatory authorities.

Source: www.drugoffice.gov.hk

Canada: Important Safety Information: NUVARING (Etonogestrel / Ethinyl Estradiol Slow Release Vaginal Ring) - New Contraindications

Date: August 1, 2014

Merck Canada Inc., in consultation with Health Canada, would like to inform you of new contraindications that have been added to the NUVARING Product Monograph (PM).

NUVARING is a combined hormonal contraceptive (CHC) indicated for conception control. The following points summarize the important changes that have been made to the PM:

- NUVARING should NOT be used by female smoker (if over age 35), or who have severe or multiple risk factors for thrombosis, including: valvular heart disease with complications, hypertension, severe dyslipoproteinemia, abnormality in proteins that regulate coagulation, diabetes mellitus with vascular involvement, or major surgery with prolonged immobilization.

- NUVARING should NOT be used by women who have experienced migraines with focal neurological symptoms, or pancreatitis associated with severe hypertriglyceridemia.

- Prescribers should consider the above new contraindications and review the updated PM when discussing treatment options with their patients.

In addition to the above contraindications, changes have also been made under WARNINGS AND PRECAUTIONS section of the PM regarding the following possible adverse events: systemic lupus erythematosus, sydenham's chorea, herpes gestationis, otosclerosis-related hearing loss, hepatocellular carcinoma, Crohn's disease, colitis ulcerosa and angioedema. Merck Canada Inc., in working with Health Canada, has updated the NUVARING PM regarding the above new contraindications and warnings which are also associated with other CHC products.

Source: healthycanadians.gc.ca

European Union: CMDh Endorses Restricted Use of Bromocriptine for Stopping Breast Milk Production

Date: August 22, 2014

The Coordination Group for Mutual Recognition and Decentralised Procedures – Human (CMDh) has endorsed by majority recommendations on the use of bromocriptine-containing medicines by mouth to prevent or suppress breast milk production (lactation) after childbirth. The CMDh agreed that the medicines should only be used for this purpose (in strengths up to 2.5 mg) when there are compelling medical reasons.

The CMDh position follows a review by the EMA's Pharmacovigilance Risk Assessment Committee (PRAC) of available data on the safety and effectiveness of bromocriptine in controlling breast milk production after childbirth, which led to these recommendations. The review was triggered by concerns in France over increased reports of rare but potentially serious or fatal side effects, particularly cardiovascular side effects (such as heart attack and stroke), neurological side effects such as seizures (fits) and psychiatric side effects (such as hallucinations and manic episodes).

Healthcare professionals are advised that the following recommendations should be borne in mind when prescribing bromocriptine for the prevention or suppression of lactation.

- Bromocriptine should only be used orally in strengths up to 2.5 mg to inhibit lactation when medically indicated, such as in case of intrapartum loss, neonatal death or HIV infection of the mother. Products with strengths of 5 or 10 mg are not indicated for such use.

- Bromocriptine should not be used for the routine suppression of lactation, nor for the relief of symptoms of post-partum pain and engorgement.

- Use is contraindicated for patients with uncontrolled hypertension, hypertensive disorders of pregnancy, hypertension post-partum and in the puerperium, a history of coronary artery disease or other severe cardiovascular conditions, or a history of severe psychiatric disorders.

- Blood pressure should be carefully monitored especially during the first day of therapy. If hypertension, suggestive chest pain, evidence of central nervous system toxicity, treatment should be discontinued and the patient evaluated promptly.

Source: www.ema.europa.eu

Canada: Summary Safety Review - Interferon-beta Products - Thrombotic Microangiopathy

Date: August 23, 2014

A safety review was initiated to evaluate the currently available information regarding the possible risk of thrombotic microangiopathy (TMA), a blood clotting disorder affecting small blood vessels, with the use of interferon-beta products.

A small number of cases of TMA after exposure to interferon-beta products have been reported in Canada and internationally. The scientific literature does not clearly explain why interferon-beta products can cause TMA.

Given the limited information available at the time of this review, Health Canada decided to continue its ongoing monitoring of adverse reaction information involving interferon-beta products, as it does for all health products, to identify and assess potential harms. Health Canada will keep Canadians informed and take action, as appropriate, if any new safety information is identified.

Source: www.hc-sc.gc.ca

Singapore: Lithium and the Risk of Hypercalcaemia and Hyperparathyroidism

Date: September 5, 2014

Health Sciences Authority (HSA) would like to share with healthcare professionals a recent advisory issued by Health Canada regarding the risk of hypercalcaemia associated with lithium therapy, which may or may not be accompanied with hyperparathyroidism.

Based on its review, Health Canada has reaffirmed that the benefits of lithium therapy in the treatment of bipolar disorder continue to outweigh the known risks of this drug. The agency had recommended reviewing patient calcium blood levels at the following times: before the start of lithium treatment, six months after initiation of lithium and subsequently on an annual basis for patients on long-term treatment. Healthcare professionals were also advised to consider measuring the PTH blood levels, if necessary, to identify or rule out hyperparathyroidism.

Hypercalcaemia and hyperparathyroidism are both known risks associated with lithium therapy. Healthcare professionals may wish to consider the above information in the management of their patients and be vigilant to possible signs and symptoms of hypercalcaemia and hyperparathyroidism in patients prescribed lithium.

Source: www.hsa.gov.sg

Updated Recommendations for Xgeva (Denosumab) Regarding Calcium Monitoring Related to the Risk of Severe Symptomatic Hypocalcemia

Date: September 16, 2014

GlaxoSmithKline Limited (GSK) updated its recommendations for Xgeva (denosumab) regarding calcium monitoring related to the risk of severe symptomatic hypocalcemia (SSH). Xgeva is indicated for the prevention of skeletal related events in adults with bone metastases from solid tumors and it is not indicated for the prevention of skeletal-related events in patients with multiple myeloma. The Xgeva prescribing information communicates the risk of severe symptomatic hypocalcemia including reports of fatal cases in the post-marketing setting. The ongoing pharmacovigilance program further characterizes the onset of hypocalcemia and the clinical manifestations, allowing GSK to provide updated recommendations for risk mitigation.

GSK advised Healthcare providers to monitor calcium levels in all patients at baseline and throughout the duration of treatment especially in the first few weeks. Other recommendations related to management of hypocalcemia related to Xgeva treatment include:

1. Pre-existing hypocalcemia must be corrected prior to initiating therapy with Xgeva.
2. Supplementation of calcium and vitamin D is required in all patients, unless hypercalcemia is present.
3. If hypocalcemia occurs, additional short-term calcium supplementation may be necessary.
4. Patients with severe renal impairment or receiving dialysis are at a higher risk of hypocalcemia.

In Hong Kong, Xgeva Solution for Injection 120mg (HK-61163) is registered by GSK and is a prescription only medicine. Regarding this issue, GSK informed DH that they will issue a "Dear Healthcare Professional Letter" to inform healthcare professionals. Besides, GSK is going to submit the application to change the package insert by including the relevant safety information. DH has not received any relevant adverse drug reaction in connection with Xgeva so far and will keep vigilant on any safety updates of the drug.

Source: www.drugoffice.gov.hk

Australia: Safety Advisory: Serotonin-blocking Medicines Used to Treat Nausea and Vomiting - Risk of Serotonin Syndrome

Date: September 23, 2014

The Therapeutic Goods Administration (TGA) advised consumers and health professionals that serotonin syndrome is a newly identified issue associated with products containing the serotonin-blocking medicines classed as 5-HT3 receptor antagonists. These medicines are used after surgery and in patients undergoing cancer treatment to prevent nausea and vomiting, by blocking serotonin from entering certain cells in the nervous system and brain.

Serotonin syndrome has been reported in patients using 5-HT3 receptor antagonists simultaneously with other serotonergic medicines. It occurs when serotonin accumulates to high levels in the body, as can happen when medicines block the chemical from entering cells. The syndrome is characterised by:

1. altered mental state, e.g. confusion, agitation, restlessness and excitement;
2. autonomic dysfunction, e.g. tachycardia, sweating, shivering, hypertension and hyperthermia;
3. neuromuscular excitation, e.g. hyperreflexia, tremor. In some cases serotonin syndrome can lead to loss of consciousness, coma and death.

Source: www.tga.gov.au

Deregistration of Rectal Suppository Containing Domperidone 10mg will Take Effect on 1 October 2014

Date: September 24, 2014

On 7 July 2014, the Pharmacy and Poisons Committee of the Pharmacy and Poisons Board decided to deregister rectal suppository containing domperidone 10mg. The deregistration will take effect on 1 October 2014 to provide a transition period for doctors to review the treatment plans for switching to suitable alternative treatment for their patients. Findings from overseas studies and the usage information of the product in Hong Kong have showed a small yet increased risk of potentially life-threatening effects on hearts.

Patients who are still using the product should contact their doctors for switching to suitable alternative treatment. When the deregistration takes effect, the product will become unregistered pharmaceutical product. The sale, offer for sale, distribution or possession of such product will constitute an offence under the Pharmacy and Poisons Regulations and the offenders will be prosecuted.

Source: www.drugoffice.gov.hk

UN Mission for Ebola Emergency Response in Accra

Date: September 30, 2014

As the number of patients with Ebola in Guinea, Liberia and Sierra Leone surpassed 6500 with more than 3000 deaths, the United Nations is increasing the momentum to curb the spread of the disease. The Special Representative of the UN Secretary-General, Mr Anthony Banbury, arrived in Accra to head the UN Mission for Ebola Emergency Response (UNMEER), which will join governments and international partners to respond to the Ebola outbreak.

Source: http://www.afro.who.int/en/media-centre/pressreleases/item/7057-un-mission-for-ebola-emergency-response-in-accra.html

Caution on the Use of Tear Gas and Pepper Spray

Date: October 1, 2014

The Pharmaceutical Society of Hong Kong and the Hong Kong Pharmacists Union wrote to Hon. Leung Chun Ying, Chief Executive, requesting that the police be cautious in the use of tear gas and pepper spray which may bring about short term or long term harm to the health of Hong Kong citizens or tourists in the neighborhood where protests may be occurring. Tear gas causes eye irritation, extreme tearing, shortness of breath, coughing, nausea and vomiting. Prolonged exposure - that is, over an hour, can cause serious damage to the eyes, skin and lungs. Pepper Spray when gets in the eyes cause pain and stinging, and temporary blindness that lasts 30 minutes or so, immediate changes in mechanical and chemical sensitivity can persist for up to a week. Tear gas and pepper spray pose a genuine risk to people who have heart conditions, asthma or other breathing problems. PSHK quoted 2 recent studies by Turkish researchers which show that tear gas can cause health problems that may last for weeks, and can also impact a wider area than targeted. Hence, they urge that the police should not use tear gas and pepper spray indiscriminately.

Source: Letters to the Chief Executive

Pharmacy & Information Technology - An interview with Ms. Chiang Sau Chu

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INTRODUCTION

Ms. Chiang Sau Chu

Many of our pharmacists, including pharmacy students at both the Chinese University of Hong Kong and the University of Hong Kong, would know Ms Chiang who has spent the past three decades working in the public health care system of Hong Kong and heard her speak at numerous different occasions, such as in the Hong Kong Pharmacy Conference, in lectures and in society meetings, etc. However, few would have the chance to hear Ms Chiang talk about what she thinks about her work and what she had been doing to help shaping the pharmacy practice in Hong Kong and how she manages her work to get so many pharmacy information technology (IT) systems developed and implemented in all of the pharmacies in the public hospital system.

In this interview, Ms. Chiang, as she is respectfully known to us, shared with us her rich experience in developing different stages of pharmacy IT systems in her current position as the Senior Pharmacist at the Hospital Authority Head Office.

BACKGROUND

Ms. Chiang obtained her pharmacy undergraduate degree in the University of Bradford, UK and completed her preregistration training in a district hospital in London before returning to Hong Kong in 1981. She started by working in an independent community pharmacy in North Point. The work in the community did not offer much challenge and as there was no news several months after the interview for a pharmacist position with the Hong Kong Government, she had to make the decision to return to UK. Whilst doing some locums in London and at the same time looking for other suitable job opportunities in the hospitals, much to her surprise, she received the offer for a pharmacist position from the Hong Kong Government. So, two months later, in July 1981, Ms Chiang returned to HK again, starting her pharmacy career in Hong Kong at the pharmaceutical headquarters of the Medical and Health Department, Hong Kong Government. In the first five years, Ms Chiang was posted to assume different duties of a pharmacist at the Pharmaceutical Headquarters as well as in the hospitals and specialist clinics. During that period, she has built up much knowledge about the functions at the central headquarter' s level and had also become familiarised about the detail operational procedures taking place in different pharmacy settings. In addition, she learned about the local cultures and how staff would behave and react towards different policies and procedures when introduced in the work environment. These proved to be very useful in enriching her work experience and had provided her the foundation when she was required to build a pharmacy system to support pharmacy workflow later in her career. In 1985, she was called back by the Chief Pharmacist to work in the Head Quarters to take up the pharmacy computerisation project which was set up at that time, based on the recommendation from the HK Government Audit Department that computerisation is the best solution to deal with the complexities in the Pharmacy management and the drugs in areas such as budget monitoring, stock control, inventory management and utilisation analysis, etc. This was a job that no one has done before but Ms Chiang was privileged to be assigned this task, partly because she had the wide experience of frontline pharmacy operations and partly because she had demonstrated an eagerness for making suggestions and improvements to the pharmacy practice in Hong Kong. This transfer from the frontline to the new job began her journey as the first pharmacy informatician in Hong Kong. Ms Chiang has introduced a whole series of IT systems all of which helped shaping the present pharmacy practice in the public hospital pharmacy system.

In the next five years until 1990, Ms Chiang was responsible for designing the entire pharmacy computerisation system including the core functions such as dispensing, labelling, inventory management, purchasing, stock taking, ward issuing, ward returns, etc.. There were many other supporting functions e.g. the Drug Master File maintenance, the contract / supplier maintenance, the user access level definition matrix, etc. The system was named the Pharmaceutical Supplies System (PHS). There were lots of other tasks to be done before the system could go live and these include users training, sites preparation, hardware delivery and installation

in addition to system testing, data conversion, system documentation, version control, user broadcasting etc. All in all it was the most challenging task with much attention from the management and staff and this had to be accomplished with periodic reports and explanation about the progress made and results achieved. It provided the best opportunity for Ms Chiang to bring in her innovations, to master her project management skills and other managerial techniques such as communication, time management, change management and more importantly Ms Chiang also had to lead, motivate and influence the pharmacists as well as the dispenser-grade staff so that the resistance to computerisation could be reduced and minimised. The PHS project went live to one site after another and as if it was not busy enough for her, between 1986 and 1987, Ms Chiang also gave birth to her daughter and son. By the year 1990, the PHS project was fully implemented in the four biggest acute general hospitals at that time, namely Queen Mary Hospital, Princess Margaret Hospital, Queen Elizabeth Hospital and Tuen Mun Hospital. Looking back, it was like a miracle that this was achieved as the project had experienced some critical points of failure and all these would not have been overcome without much management efforts and team spirit.

In the year 1990, upon the completion of the project implementation, Ms Chiang was transferred back to work in the specialist clinic where she thought she could be given the peaceful period when she could spend more time looking after her children. This lasted for only one year until the Hospital Authority was established in 1991, when a few serious medication incidents occurred at the Prince of Wales Hospital. Ms. Chiang was again called back by the Chief Pharmacist to return to the Hospital Authority Head Office and to serve in the working group which was set up to investigate into these incidents. This transfer was so far the last transfer in Ms. Chiang's career and until now Ms Chiang is still positioned at the Chief Pharmacist's Office to serve the frontline from the central level.

PJ: What are your roles and responsibilities in the Chief Pharmacist's Office now?

Ms. Chiang: I have a team of about twelve Pharmacists, a Chief Dispenser and a few Senior Dispensers and our team is responsible for designing, developing and supporting all the pharmacy IT systems used in the Hospital Authority. We work closely with hospital IT teams to make sure the smooth running of all of our systems which have now extended the scope and functional use by doctors and nurses too.

PJ: Have you encountered any challenge when developing the pharmacy IT systems?

Ms. Chiang: As the pharmacies of all hospitals utilise the same pharmacy system and also the physicians use the same order entry system in the Hospital Authority, the biggest challenge is to design a standardised system which can be used by both the physicians and pharmacies at all hospitals. We have included general functions to meet the core needs of both physicians and pharmacies and there are also special functions to cater for special needs that were added later on. Usually, we aim to make the functions user-friendly that can suit most if not all personnel.

Another challenge in the beginning is the staff's resistance to change and to accept and use computerised systems in replacement of their traditional ways of work. However, this is no longer so, as our staff has gradually come to acknowledge that computerised systems tremendously facilitated their work to the extent that these have become indispensable.

PJ: How do you motivate the staff to use IT system?

Ms. Chiang: First and foremost, we always need to think from the staff's perspectives and put ourselves in their shoes. We need to familiarise ourselves with the frontline operations so as to design functions to meet their actual operational needs. We also need to design systems that will simplify the workflow procedures and above all, do not make the system complicated to use. We also need to take safety into account. When the staff expresses their concern about the system, we have to listen and act promptly giving them a quick response with the corresponding system design details and explanations about the system logics and rationalities and sometimes limitations and constraints. So far, with the implementation of so many different systems, such as the Express Dispensing System (EDS), the Medication Order Entry System (MOE), the Corporate Drug Dispensing History (CDDH) and the Pharmacy Data Query Template, our staff now remain very convinced that IT systems can definitely bring positive impacts in their daily pharmacy practice. Hence, we do not have much resistance for our staff to use IT systems. In fact, our staff is now eager for more frequent and faster release of newer systems as they know that each version upgrade would bring them enriched features to help improve safety, quality and efficiency in their daily work.

PJ: Do you think motivation is an essential quality of a pharmacist?

Ms. Chiang: I think everybody needs some motivation at times and also every pharmacist should share the responsibility to initiate some form of improvement to keep herself / himself motivated. The way I see it is when we seek to continuously improvise innovative solutions that are well accepted, this improvement process acts as a spur and can motivate us to strive for further improvement.

PJ: You always travel to different places and gain exposure to their different pharmacy systems. Do you think there is any system suitable for introduction to Hong Kong?

Ms. Chiang: In my opinion, the pharmacy practice and our systems in Hong Kong is at a cross road. We need leadership to guide our pharmacists and dispenser-grade staff to make a breakthrough in what we are doing and how we do our things. Therefore, I think

there is a need for both pharmacy students and pharmacists to start by learning how to lead himself / herself and then to lead a team. In the long run, these leaders can guide the future pharmacy practice in order to achieve breakthroughs in many different areas such as our IT and automation systems, our clinical pharmacy services and in research and other fields of pharmacy practice.

When compared to overseas countries, the number of pharmacists in Hong Kong is relatively small. Besides, the Hospital Authority takes care of the majority portion of the healthcare services in Hong Kong. This has exerted a heavy burden on the pharmacies in the public health system, forcing them to focus a significant portion of their work on dispensing activities. Therefore, there is a heavy demand for good IT pharmacy system and automatic dispensing systems in Hong Kong in order to ease the burden of dispensing work. It has been proven that automation can make the dispensing process more efficient so that more pharmacists' time can be spared for clinical patient care activities.

PJ: What is the future direction of system development at the Chief Pharmacist's Office?

Ms. Chiang: Our team aims to continue to design new systems for the frontline pharmacy. There are currently four big projects. Firstly, we have the closed-loop In-patient Medication Order Entry (IPMOE) system which has been gradually implemented in Princess Margaret Hospital since April 2013 and Tseung Kwan O Hospital since May 2014 and will be implemented in Prince of Wales Hospital in July 2014. In three to four years' time, we plan to promote this system to a total of fourteen hospitals and we also aim to optimise some of its functions and enrich its features. The initial implementation goals of this system are less medication related incidents and more streamlined pharmacy in-patient dispensing work flow and more efficient and timely drug administration rounds.

Secondly, we aim to introduce more enhanced features to our existing out-patient dispensing system such as for the system to differentiate medication related changes for our out-patients in their new prescription orders when compared to the previous or old medications orders so that our pharmacy staff can focus on counselling our patients on the changes that have been made, so that pharmacists can better utilise their time for more patient-centred counselling.

Thirdly, the order receipt processes and pharmaceutical product supply chain of our pharmacies have been modernised by introducing "Wi-fi" scanners and barcode system to allow track and trace of the products when these are delivered from the distributors or suppliers into our pharmacy stores.

To acknowledge the good work that we have accomplished, the Hospital Authority has received the global recognition, which is the Provider Implementation Best Case Study Award in April from Global GS1. The next goal of this project is to see how we can extend the tracking function to the drug dispensing of ward stock and to the in-patient areas.

Lastly, we are also exploring the use of some automatic dispensing machines to our In-patient Medication Order Entry System to provide bar coded unit dose dispensing which will help to improve medication and patient safety at the clinical areas.

PJ: What advice would you offer to new pharmacy graduates and existing pharmacists in Hong Kong?

Ms. Chiang: I note that new pharmacy graduates and majority of the pharmacists in our public health system are very hardworking and they try their best in their work. It is good that we do our part well. However, sometimes our pharmacy staff focuses too much on the one part of the pharmacy work, such as dispensing process so much so that they have become some form of automatic dispensing machines, which means they do their job without thinking. I note that they work very hard aiming to complete the dispensing of all the prescriptions instead of checking to see if the use of the medications is necessary and rational. Therefore, my opinion is that there is a need to critically review what the role of our pharmacists and our pharmacies is and how the system should change to meet the future demands of our ageing population. Otherwise, I have doubts about the sustainability of our system.

PJ: Is there anything that you would like to share with the readers?

Ms. Chiang: This journal is the only professional publication for the pharmacy profession in Hong Kong. It is rather regretful that we are not able to attract many people to submit articles for the journal. Therefore, I hope that more people will support this journal by not only reading, but also making more contributions. This may include to write stories and articles about the hospital pharmacy practice in Hong Kong.

Last but not least, Ms. Chiang shared that she has just become a grandmother. Congratulations to Ms. Chiang and her family!

Author's background

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1. Triplitt C, et al. Vasc Health Risk Manag. 2010; 6: 671–690 2. Oseni Hong Kong Product Monograph 3. White WB et at. N Engl J Med 2013; 369:1327-1335 4. Dormandy JA, et al. Lancet. 2005; 366(9493):1279-1289. 5. Hanefeld M, et al. Curr Med Res Opin 2006; 22(6):1211-1215. 6. Del Prato S, et al. Diabetes Obes Metab. 2014 Aug 8.

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Overview of Medications for Solid Organ Transplantation

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ABSTRACT

Solid organ transplantations (SOT) always involve complex immuno-responses not only because of potential rejection of the new organ but the transplanted patients are also more prone to viral (e.g. cytomegalovirus, herpes simplex virus), bacterial and fungal (e.g. candida, pneumocystitis) infections. Hence, anti-microbial treatment is often warranted in the transplanted recipients. As a result, polypharmacy is applied in these patients such that close monitoring in pharmacotherapy is necessary. Clinical pharmacists, who are specialized in pharmacotherapy, therefore, have a critical role to address drug-related therapeutic issues in this population of patients and have a better understanding about the pharmacotherapy of SOT. The aim of this article is to discuss the basic immunology of organ transplant rejection, the commonly used transplantation medications as well as other post-transplantation medications. The emerging trend in immunosuppressive therapy is also reviewed.

Keywords: *Solid organ transplantation, transplantation, immunosuppressive therapy, immunosuppressant, transplant rejection*

INTRODUCTION

Solid organ transplantations (SOT) always involve high degree of complexity of immuno-responses not only because of high potential rejection of the new organ but transplant patients are also more prone to viral (e.g. cytomegalovirus, herpes simplex virus), bacterial and fungal (e.g. candida, pneumocystitis) infections, anti-microbial treatment is often warranted in the transplant recipients.[1] The most frequently performed SOTs are those of the visceral organs (kidney, liver and pancreas) followed by those of the thoracic organs (heart and lung).[2] In Hong Kong, based on the data from the Hospital Authority, SOT (both cadaveric and living) has remained at a steady high demand since 2001 (Table 1). These different parameters determine the choice of a therapy for most types of end-stage organ failure.[3] To understand how transplantation medication works, it is essential to review the basic immunology of transplantation.

Table 1. Number of organ/tissue donation & patient waiting for transplantation under Hong Kong Hospital Authority (1.1.2004-31.12.2013)

Organ/Tissue	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	No. of patient waiting for transplantation (as at 31.12.2013)
Kidney donation											
Deceased donor	44	50	53	58	65	87	74	59	84	70	1991
Live donor	6	8	13	8	12	8	7	8	15	12	
Liver donation											
Deceased donor	20	24	23	26	26	43	42	30	45	38	120
Live donor	56	38	48	41	42	41	53	44	33	34	
Heart donation	7	8	7	5	6	10	13	9	17	11	17
Double Lung donation	0	2	1	1	1	2	2	1	3	2	18
Single Lung donation	0	0	0	0	0	0	0	0	0	2	
Cornea donation (piece)	230	214	244	198	211	203	250	238	259	248	500
Skin donation	30	13	8	13	19	17	23	21	6	4	Uncertain
Bone donation	4	3	3	1	1	0	6	0	3	3	Uncertain

BASIC IMMUNOLOGY OF TRANSPLANTATION

While the immune response to a transplanted organ consists of both cellular (lymphocyte medicated) and humoral (antibody mediated) mechanisms, T cells are central in the rejection of grafts. The rejection mechanism can be divided into a sensitization phase and an effector phase (Fig. 1). During the sensitization phase, the CD 4 and CD 8 T cells, via their receptors, can recognize the allo-antigens expressed on the cells of the foreign graft where two signals are needed for recognition of an antigen.[4] In the effector phase, the non-immunological "injury response", that is, ischemia will trigger the transplant recipient's immune system to identify the transplanted tissue as "foreign" and lymphocytes will become attached to human leukocyte antigen (HLC) on the tissue's surface. Subsequently this will trigger signals 1 and 2. These signals then activate the nucleus of the T helper cell via the intracellular calcineurin pathway to produce mRNA and DNA and then increase the production of interleukin 2 and trigger signal 3. In turn, interleukin 2 induces cellular proliferation among nearby target lymphocytes. The net result is Signals 1 & 2 lead to signal 3 which causes the production of cytotoxic T cells and Antibody producing B cells and complement, which will attack the transplanted tissue, resulting in rejection.[4]

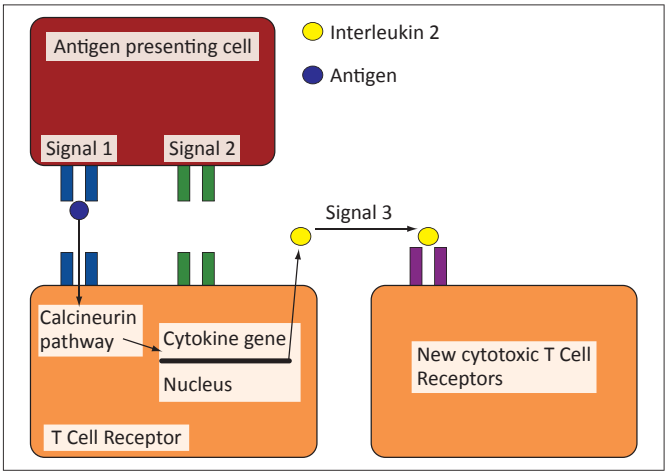

Figure 1: *The immunology of rejection.*

THE COMMONLY USED TRANSPLANT MEDICATIONS

In order to prevent rejection, an "ideal" immunosuppressant (Fig. 2) should selectively inhibit alloantigen immune response, prevent allograft rejection, be free of adverse events, have few drug interactions, and be lower in cost.[5] Unfortunately, these "ideal" medications do not exist. Immunosuppressants are generally used for several purposes: (1) induction therapy (2) maintenance therapy, and in unfortunate incidents, for (3) rejection therapy.[6]

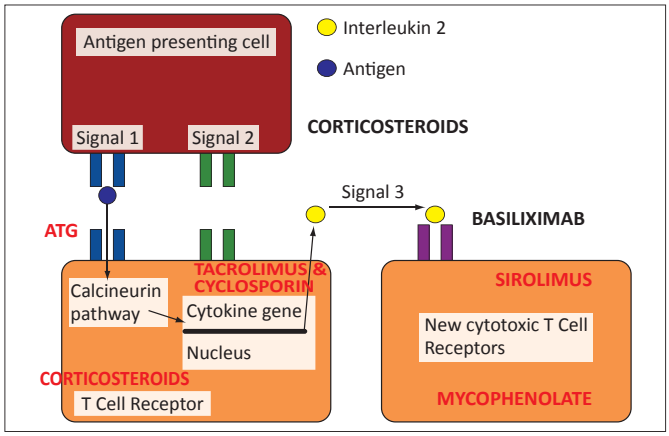

Figure 2: *The basic mechanisms of commonly used immunosuppressants.*

The induction phase refers to the administration of selective, potent agents during the initial period of allograft placement to decrease the risk of acute rejection. This may allow an overall reduction in the intensity of early post-transplant maintenance immunosuppressive therapy. Whether or not an induction therapy is included in the initial regimen to prevent transplant rejection varies with the type of transplant and individual centers. On the other hand, the maintenance phase signifies the long-term immunosuppressive regimen, typically initiated within the early postoperative period. In general, it combines two or more medications from different drug classes with different mechanisms of action. In the case of organ rejection, the pharmacologic regimens used vary depending on rejection type (acute vs. chronic), rejection severity (mild, moderate or severe), and the immune system involved (T and/or B-lymphocytes). For moderate to severe rejections, agents typically classified as T- or B-cell induction therapies (e.g. anti-thymocyte globulin, OKT3 or basiliximab) are often used, together with corticosteroids. For mild rejection episodes, corticosteroid boluses, possibly together with an increase in maintenance immunosuppressive medications, are often used.

Calcineurin Inhibitors (CNIs) – Cyclosporine& Tacrolimus

Cyclosporine

Its mechanism of action is to block the production of IL-2 by T-helper (Th) cells through inhibition of their messenger RNA. The initial oral dose usually starts with 5-10mg/kg/day in two divided doses (around 30% bioavailability), reduced to a maintenance dose of as low as 3mg/kg/day.[1] Individual patient handling of cyclosporine is very variable and the dose must be tailored depending on the type of transplantation and according to serum cyclosporine levels. The post-transplant dose should be adjusted according to the trough levels.[6] The trough level should be taken before the morning dose every 2-3 days until therapy is stabilized, then monthly, or 2-3 days after any dosing change. Target levels may be reduced due to nephrotoxicity or infections. The common toxic effects include nephrotoxicity and hypertension, as well as hirsutism, acne and gum hypertrophy.

Practice points required for Therapeutic Drug Monitoring (TDM) of immunosuppressant[7,12]

- Concentration monitoring is recommended in transplant patients.
- Cyclosporine whole blood trough concentrations are used to monitor efficacy and toxicity.
- The target concentration depends on several factors including the indication, whether adverse effects have occurred and the assay method. In transplant patients, it also depends on the time since transplant, use of other immunosuppressants, and whether rejection has occurred.
- Whole blood concentrations are used; sampling approaches include a single trough concentration (C0), a single concentration 2 hours after a dose (C2) or multiple timed samples for calculation of area under the curve (AUC).
- Single trough concentration (C0): Blood samples are obtained 12 hours after the last administered dose and target typically ranges from 50 to 400 ng/mL depending on the organ transplanted and the elapsed time since transplantation and should be individualized.
- 2 hours after a dose (C2): Target concentrations range from 800 to 2000 ng/mL. 2-hour post-dose concentration correlates with efficacy and safety better than C0.
- After a dose change, it may take 3–5 days to reach a stable concentration.

Tacrolimus (FK506)

This is a macrolide immunosuppressant that inhibits T-cell activation, T-helper cell activation and T-helper cell-dependent B-cell proliferation, as well as the formation of lymphokines such as IL-2, IL-3 and beta-interferon through similar mechanisms to cyclosporine. An initial dose usually starts with 0.1mg/kg/day in two divided oral doses (around 20% of bioavailability) and again the dose is adjusted based on the blood levels.[13] In practice, 12-hour trough levels are routinely measured for tacrolimus, with a target level of 5-20 micrograms/L depending on the time post-transplant and the type of transplant undertaken.[6] As with cyclosporine, the side-effects of the drug include nephrotoxicity and hypertension; however it does not cause the other more cosmetic side-effects of cyclosporine. Indeed, studies comparing cyclosporine with tacrolimus as primary immunosuppressant following renal transplantation have shown tacrolimus to more potent than cyclosporine (lower incidence of acute allograft rejection) for similar degree of adverse effects.[5]

Regarding the important drug interactions for CNI, sub-therapeutic levels of both cyclosporine and tacrolimus will lead to graft damage or graft loss. As the CNIs are extensively metabolized by the hepatic CYP3A4 enzymes, numerous clinically significant interactions may result if CNIs are used concurrently with potent CYP3A4 inducers such as rifampicin, carbamazepine, phenytoin. Also, increased toxicity will be found if CNIs are used with potent CYP3A4 inhibitors, for example, other nephrotoxic agents (NSAIDs, gentamicin), macrolide antibiotics, azole antifungals, calcium channel blockers, or grapefruit juice.[5]

Practice points required for Therapeutic Drug Monitoring (TDM) of immunosuppressant [7,12]

- Tacrolimus whole blood trough concentrations are used to monitor efficacy and toxicity
- Therapeutic trough levels (C0): Blood samples are obtained 12 hours after the last administered dose. Target typically ranges from 5 to 20 ng/mL depending on organ transplant and elapsed time since transplantation should be individualized for each patient.
- Whole blood for tacrolimus levels should NOT be drawn from the same line through which it was infused. Results will show falsely increased levels since tacrolimus will bind to line sets.
- After a dose, brand or formulation change, it may take 3–5 days to reach a stable trough concentration.

Mycophenolate mofetil (MMF)

MMF is a prodrug of mycophenoic acid and acts by inhibiting the intracellular de novo pathway for purine synthesis. Most cells also possess a salvage pathway, but T lymphocytes do not, thereby rendering them unable to synthesis purines, which in turn prevents successful replication. The IV dose is similar to the oral dose (either 1g bd or 720 mg bd) and the administration of the drug should be given as 12 hourly dosing with food for the same time each day.(7) The main side effects of the drug are gastrointestinal upsets and increased susceptibility to infections, especially viral infections. Another formulation, mycophenolate sodium EC, is now available and it is purported to have a lower incidence of gastrointestinal side-effects, though evidence does not support this claim. Routine monitoring of the drug level is not common in real practice, however, complete blood count, especially neutrophil count, should be monitored because of the risk of neutropenia. If the patient develops neutropenia (neutrophil count $<1.3x10^3$ micro/L), it may be appropriate to interrupt or discontinue MMF therapy.[7]

Practice points required for Therapeutic Drug Monitoring (TDM) of immunosuppressants [7,12]

- Blood concentrations can be monitored but it is not done routinely.
- May be considered in some cases (e.g. high immunological risk, gastrointestinaladverse effects, change in drug regimen or brand, and renal impairment).
- Plasma concentrations of mycophenolic acid are used.
- Refer to the relevant specialist unit for specific recommendations.
- After a dose change, it may take 3–4 days to reach a steady state.

Corticosteroids

Corticosteroids have multiple immunosuppressive mechanisms, including alteration of T-cell proliferation, inhibition of cytokine production, suppression of macrophage function, reduction of adhesion molecule expression and induction of lymphocyte apoptosis. They are commonly used as adjunct to other immunosuppressants as induction and maintenance therapy, and also in high doses in rejection therapy. The initial dose is usually started intra-operatively with IV methylprednisolone in the anaesthetic bay and then stepped down when appropriate to oral prednisolone for maintenance. Adverse effects are commonly encountered and include cushingnoid appearance, hypertension, hyperglycaemia, weight gain, increased susceptibility to infection and personality changes. Long term complications include skin and muscle atrophy and avascular necrosis of bone.

Sirolimus

Sirolimus is chemically related to tacrolimus but is a novel immunosuppressive agent that inhibits T-cell proliferation by inhibiting cytokine-mediated signal transduction pathways. It has been used in combinations with cyclosporine and prednisolone to lower the incidence of early acute rejection. The side effects include hyperlipidaemia, anaemia and severe aphthous ulceration. However, its place in therapy has not been defined by NICE guidelines as in the treatment of chronic rejection.[9]

Practice points required for Therapeutic Drug Monitoring (TDM) of immunosuppressants [7,12]

- Whole blood concentrations should be monitored and it depends on assay method and are not interchangeable.
- Target typically ranges from 3 to 12 ng/mL, depending on organ transplanted and elapsed time since transplantation should be individualized.
- The half-life of sirolimus is about 62 hours, thus steady state is not achieved for several days where sirolimus trough levels are typically checked on weekly basis.
- It is recommended that sirolimus be taken 4 hours after the administration of cyclosporine modified because of an increase in the maximum concentration (Cmax) and the AUC of sirolimus when both drugs are taken concomitantly. This interaction is not seen when sirolimus is used concomitantly with tacrolimus.

Monoclonal Antibodies

These agents bind to the CD 25 antigen on human T lymphocytes to prevent them from expressing IL-2, thereby inhibiting the activation of T-cytotoxic cells and hence effectively hiding the graft from the sponsor's immune system, resulting in a reduced incidence of acute rejection.

Basiliximab

This is a chimeric monoclonal antibody (70% human, 30% murine). It binds to the interleukin-2 (IL-2) receptors and functions to inhibit IL-2-mediated T-lymphocyte activation to prevent graft rejection. It is used as induction therapy to prevent transplant rejection.[7]

The dose for induction immunosuppression usually starts with 20mg IV on day 1 and then 20mg IV on day 4. The common side effects may include: hypertension, peripheral oedema, fever, headache, insomnia, pain, acne, hypercholesterolaemia, hyper-/hypo-kalaemia, hyperglycaemia, hyperuricaemia, hyperphosphataemia, abdominal pain, constipation, dyspepsia, nausea, vomiting, UTI, anaemia, tremor and upper respiratory infection. Although the list of side-effects may appear lengthy, not all are routinely experienced in clinical practice.

Muromonab-CD3 (OKT-3)

OKT3 is a murine monoclonal antibody used only for the treatment of severe acute rejection that is corticosteroid-resistant. It binds to CD3-receptors on T cells, forming an OKT-3/CD3 complex, which then undergoes endocytosis, removing the receptor from the cell surface. Due to its low usage and

the association with serious adverse reactions, such as the cytokine-release syndrome, the manufacturer has discontinued its production.

Anti-thymocyte globulins (ATG horse or rabbit)

ATG removes circulating T lymphocytes and blocks their formation and proliferation in response to antigenic stimuli. It reacts with a wide variety of receptors on T cells. Before the advent of the newer monoclonal antibodies, a 10-day course of low-dose ATG was given as induction therapy after a "high-risk" transplant, but its use now tends to be reserved for salvage therapy in patients whose acute vascular rejection episodes are not responding to high-dose steroids. The common side effects of the drug are fever, arthralgia, rash, leukopenia, thrombocytopenia, diarrhea, nausea and vomiting.

Other Post-Transplantation medication

All transplant patients need to be followed up closely after discharge from hospital for allograft complications after transplant and rehabilitation. Besides allograft rejection, complications usually relate to the risk of opportunistic infections. For routine prevention of infections, prophylaxis against Pneuomocystisjiroveci PCP pneumonia, anti-fungal (i.e. Candida +/- Aspergillosis), and anti-viral against CMV are usually necessary. The risk of PCP pneumonia is around 5-14% for patients not receiving PCP prophylaxis. The risk is directly related to the degree of immunosuppression. A dose of 480 or 960 mg daily is usually sufficient.[9] For anti-fungal prophylaxis, the triazole anti-fungals such as fluconazole at 200 to 400 mg daily are used and well tolerated. For cytomegalovirus (CMV) mismatch (donor positive, recipient negative) cases, the incidence of CMV infection is very high and may be reduced by prophylactic therapy with a selection of prophylactic therapies. (Table 2)

Table 2. Therapy options for Cytomegalovirus Infection in adult transplant recipients.[12]

	Usual Dose	Common Adverse Events
Valganciclovir (Valcyte)	450-900 mg/day orally	GI upset Headache Myelosuppression
Acyclovir (Zovirax)	800 mg 4 times/day	GI upset Headache
Ganciclovir (Cytovene)	5 mg/kg/day intravenously 1000 mg 3 times/day	GI upset Myelosuppression Rash
Valacyclovir (Valcyte)	1-2 g 4 times/day	GI upset Headache Myelosuppression
Cytomegalovirus immunoglobulin (CytoGam)	Maximal total dose per infusion of 150 mg/kg initiated within 72 hours of transplantation. Follow-up doses and frequency vary by organ type	Fevers and chills Flushing GI upset

GI = gastrointestinal

EMERGING TRENDS IN IMMUNOSUPPRESSIVE THERAPY

Not surprisingly, some general trends have been occurring over the last 15 years for immunosuppressants. Firstly, it is the use of generic immunosuppressants. Prograft and CellCept are the first generic formulations that were approved in 2009 and according to the FDA, a generic drug can be substituted for the innovator (brand) product if it is pharmaceutically equivalent and bioequivalent.[11] Secondly, the use of antibody-based induction therapy has been increasing, such as rabbit antithymocyte globulin (Thymoglobulin), monoclonal anti-IL-2-receptor antagonists (daclizumab&basiliximab) and the newer alemtuzumab. Though OKT3 & ATGAM have been used, the practice is probably being transformed to favourthe use of antibody-based regimens for most organs. The reason is that thymoglobulin and ATGAM are derived from rabbits & horses, respectively; thus theycan cause anaphylaxis, whereas the monoclonal antibodies do not. Fourthly, for maintenance therapy, tacrolimus and MMF are the most common first-line combination these days while the use of cyclosporineand azathioprine is falling.[11] Monotherapy with a single immunosuppressant is still rarely used. When it comes to steroid, in some transplant groups, steroid-avoidance or withdrawal is getting more common, for example kidney transplants from living donors.[5] However, the use of corticosteroid is still a large part of immunosuppression for lung & heart transplants & any organ rejection.

Author's background

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References

1. Stemer G and Lemmens-Gruber R.(2010). Clinical pharmacy services and solid organ transplantation: a literature review. *Pharm World Sci.* **32**:7–18.
2. Lauchart W, Gubernatis G, Blu¨mke M. (2005). Transplantation medicine. *Intensivmedizin.* **42**(6):481–8.
3. Official website of Department of Health http://www.organdonation.gov.hk/eng/statistics.html (accessed on 2/8/2014)
4. Malhotra P., Malu S. and Kapur S. et al. Immunology of Transplant Rejection. http://emedicine.medscape.com/article/432209-overview#aw2aab6b5 (accessed on 2/8/2014)
5. Marcen R. (2009). Immunosuppressive drugs in kidney transplantation. *Drugs.* 69:2227-43.
6. Shehata N, Palda V.A., Meyer R.M. et al. (2010). The use of immunoglobulin therapy for patients undergoing solid organ transplantation: an evidence-based practice guideline. *Transfus Med Rev.* 24:S7-S27.
7. Adelaide, S.Aust. : Australian Medicines Handbook, 2014 Edition.
8. Gelder T, Le Meur Y, Shaw LM, et al (2006). Therapeutic drug monitoring of mycophenolate mofetil in transplantation. *Ther Drug Monit.* 28:145-154.
9. NICE guideline: Renal transplantation-immuno-suppressive regimens (adults) (TA85). Accessed on 12/12/13 http://www.nice.org.uk/guidance/TA85
10. Fishman J.A. (2007). Infection in solid organ transplant recipients. *N Engl J Med.* 357:2601-14.
11. Alloway R.R. Isaacs R., Lake K. (2009) Report of the American Society of Transplantation Conference on Immunosuppressive Drugs and the Use of Generic Immunosuppressants. *Am J Transplant* 3:1211-5.
12. Kaiser TE. Solid Organ Transplantation. ACCP Updates in Therapeutics (2012): The Ambulatory Care Pharmacy Preparatory Review and Recertification Course.

Questions for Pharmacy Central Continuing Education Committee Program

(Please be informed that this article and answer sheet will be available on PCCC website concurrently. Members may go to PCCC website (www.pccchk.com) to fill in their answers there.)

2 CE Units

Overview of Medications for Solid Organ Transplantation

1. ***What is the highest number of patient waiting for organ/ tissue donation as at 31.12.2013 based on the statistic of Hospital Authority?***
   A. Kidney donation
   B. Liver donation
   C. Heart donation
   D. Lung donation

2. ***The major molecule responsible for rejection of transplant is?***
   A. B cells
   B. T cells
   C. MHC molecules
   D. Antibodies

3. ***Which one of the following options best describes the way in which tacrolimus exerts its pharmacologic action?***
   A. Forming a complex with FK-binding protein 12, which binds to and inhibits calcineurin phosphatase and ultimately T-cell inhibition.
   B. Forming a complex with cyclophilin, this binds to and inhibits calcineurin phosphatase and ultimately T-cell inhibition.
   C. Forming a complex with FK-binding protein 12, which binds to and inhibits calcineurin phosphatase and ultimately B-cell inhibition.
   D. Forming a complex with cyclophilin, this binds to and inhibits calcineurin phosphatase and ultimately B-cell inhibition.

4. ***Which one of the following options best describes the way in which sirolimus exerts its pharmacologic action?***
   A. Forming a complex with FK-binding protein 12, which binds to and inhibits calcineurin phosphatase and ultimately T-cell inhibition.
   B. Forming a complex with cyclophilin, this binds to and inhibits calcineurin phosphatase and ultimately T-cell inhibition.
   C. Forming a complex with FK-binding protein 12, which binds to and inhibits calcineurin phosphatase and ultimately B-cell inhibition.
   D. Forming a complex with mTOR, which binds to FK-binding protein 12 and ultimately inhibits T-cell proliferation.

5. ***Avascular necrosis of bone associated with immunosuppression therapy is mostly due to which of the following medications?***
   A. Prednisolone
   B. Cyclosporine
   C. Azathioprine
   D. OKT-3

6. ***Which of the following is the common side effect of mycophenolate mofetil (MMF)?***
   A. Pulmonary fibrosis
   B. Seizures
   C. GI upset
   D. Headache

7. ***What common opportunistic infection is for the recipient at risk of developing secondary to immunosuppression?***
   A. Legionella
   B. Cytomegalovirus
   C. Tuberculosis
   D. HIV

8. ***What is the therapeutic indication for acyclovir therapy for patient after solid organ transplantation?***
   A. Varicella virus prophylaxis
   B. Cytomegalovirus prophylaxis
   C. PCP treatment
   D. No appropriate indication exists

9. ***Which of the following is a common adverse effect of ganciclovir?***
   A. Neutropenia
   B. Pulmonary fibrosis
   C. Seizures
   D. Bradycardia

10. ***Which of the following is truth about the chemical nature of basiliximab?***
    A. Murine monoclonal antibody
    B. Chimeric monoclonal antibody
    C. Humanized monoclonal antibody
    D. Equine monoclonal antibody

Answers will be released in the next issue of HKPJ.

CE Questions Answer for 212(D&T)

MC Questions for PJ article

1. B 2. C 3. C 4. C 5. D 6. D 7. A 8. B 9. A 10. D

Cloning and Expression of a Surface Antigen of *Orientia tsutsugamushi* in *E. coli*

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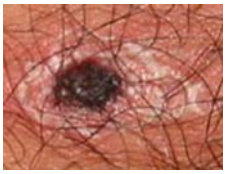
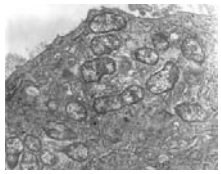
ABSTRACT

Scrub typhus or Tsutsugamushi Disease is an acute and febrile disease caused by infection with *Orientia* (formerly *Rickettsia*) *tsutsugamushi*. This disease causing significant health problem for military and civilian and is confined to a definite geographic region called 'tsutsugamushi triangle'. Larvae (chiggers) of trombiculid mites (Leptotrombidium) are vectors of scrub typhus fever transmitting the disease to human after a bite by them. In this project, part of 110 kDa surface antigen of *O. tsutsugamushi* was expressed by recombinant DNA method. The protein antigen coding sequence was amplified by PCR and ligated to pThioHis-B expression vector and was expressed as a fusion to HisPatch-thioredoxin in TOP 10 *E. coli*. The expressed fusion protein, which was overexpressed in 4.5 h after IPTG induction, was 61 kDa in size; in which 16 kDa was belonged to HP thioredoxin and 45 kDa was the antigenic polypeptides. The fusion protein could be used as a recombinant subunit vaccine for immunization against the *O. tsutsugamushi*.

Keywords: *Scrub typhus, Orientia tsutsugamushi, trombiculid mites, pThioHis-B expression vector, recombinant subunit vaccine, antigenic polypeptide*

INTRODUCTION

Scrub typhus or Tsutsugamushi Disease is an acute, febrile and systemic human disease caused by infection with *Orientia* (formerly *Rickettsia*) *tsutsugamushi* and transmitted by the bite of the larval stage of a trombiculid mite.[1] Hence, it is regarded as zoonosis. Primary lesion often develops at the site of the bite and subsequent symptoms resemble those of epidemic typhus. Incubation period after infection is about 1-3 week. The disease is potentially lethal for military solders and civilian in the geographic region called 'tsutsugamushi triangle' which extends from northern Japan and far eastern Russia in the north, to northern Australia in the south, and to Pakistan and Afghanistan in the west (Fig.1). In this region, about 1 billion people are believed at frequent risk.[2, 3]

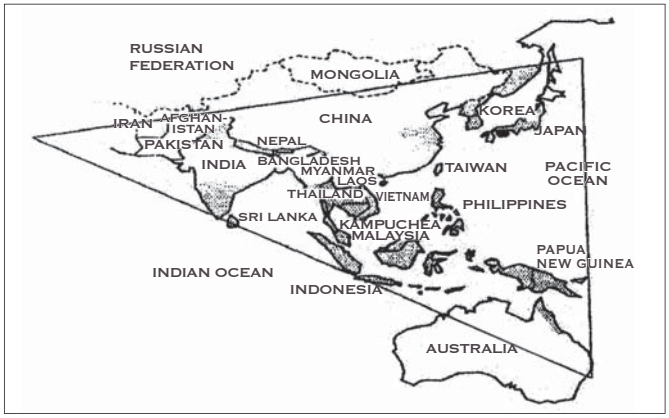


Figure 1. *Geographic distribution of Tsutsugamushi Disease.*

The principal ecologic features that distinguish scrub typhus from other enzootic rickettsiosis are related to the localization of the disease to definite geographic regions and the bionomics of the infection cycle that involves trombiculid mite vectors and their vertebrate hosts.[4] Scrub typhus caused by *O. tsutsugamushi* is commonly found in rodents and tree-shrews. Hence, Larvae (chiggers) of several species of trombiculid mites (Leptotrombidium), such as *L. denliense* and *L. fletcheri*, are vectors of scrub typhus fever. The vector lives in damp soil or detritus, in the wild and semi wild overgrown country where rodents inhibit and they satisfy its biological requirement. *O. tsutsugamushi* maintained in nature by highly efficient trans-ovarial transmission in trombiculid mites and is transmitted to humans by bites of chigger.[5, 6]

O. tsutsugamushi, an obligate intracellular bacterium, enters mammalian cells through a process of attachment and induced phagocytosis.[7] It usually infects endothelial cells, macrophages and polymorphonuclear leukocytes (PMNS)[8] in patients or in experimental animals (Fig. 2). After induction of phagocytosis, it is taken into a phagosome.[7, 8] Once escaped from the phagosome and entered the glycogen rich cytoplasm,[9] it usually propagates by binary fission, thereby causing injury to the cell. It is released by budding and covered with host cell membrane.[7]

The typhus and spotted fever group species are belonged to the genus Rickettsia.[10] *O. tsutsugamushi* is a gram-negative bacterium. The ultrastructure of its cell wall differs significantly from those of its closest relatives. In contrast to other gram-negative bacteria, it has neither lipopolysaccharide nor peptidoglycan layer.[11-14]

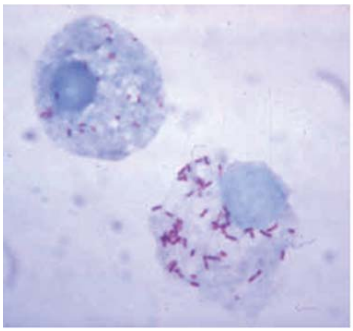


Figure 2. *Gimenez stain of tick hemolymph cells infected with Orientia tsutsugamushi. (CDC) O. Tsutsugamushi is a very small bacterium that must live inside the cells of its hosts. They are difficult to see in tissues by using routine histologic stains and generally require the use of special staining methods.*

The major surface protein antigen of *O. tsutsugamush*i is a variable 56-kDa protein which accounts for 10 to 15% of its total protein.[10,15] This family of polypeptides contains strain specific regions.[16,17] Many serotype-specific monoclonal antibodies to *Orientia* react with homologs of the 56-kDa protein Sera from most scrub typhus patients react strongly with this protein suggesting that it is a good candidate for use as a diagnostic antigen.[18] The only other polypeptide with strain–specific characteristics is the 110-kDa polypeptide antigen but the strain specific epitopes have not been identified on this particular polypeptide.[19,20]

Antigenic heterogeneity amongst *O. tsutsugamushi* strains is so pronounced that whole cell vaccines prepared against one strain generally fail to protect mice against infection from others. Although immunization with irradiated or inactivated *O. tsutsugamushi* induces protective immunity against homotypic challenging infections, antigenic diversity prevents their use in humans.[21,22] Because of the diversity, an ideal vaccine against scrub typhus is most likely a recombinant protein derived from various strains. In this project, we report a gene of interest was fused to a modified version of the thioredoxin (trxA) gene on pThioHis-B expression vector and expressed as a fusion protein in *E. coli*. Through this strategy, gene product of clone of a surface antigen from the pathogenic organism was successfully expressed.

MATERIALS & METHODS

Preparation of Competent TOP 10 *E. coli* by Inoue Method

Preparation of Inoue Transformation Buffer

PIPES 0.5 M (pH 6.7) (piperazine-1,2-bis[2-ethanesulfonic acid]) was first prepared by dissolving 3.78 g of PIPES in 25 ml Milli Q water and the pH was adjusted to 6.7 by 5 M KOH. It was stored frozen at -20°C until used. Inoue transformation buffer was prepared by dissolving 0.544 g manganese chloride $MnCl2{\bullet}4H_2O$, 0.11 g calcium chloride $CaCl2{\bullet}2H_2O$, 0.93 g potassium chloride KCl and 1 ml PIPES (0.5 M, pH6.7) in 50 ml Milli Q water to final concentration 55 mM, 15 mM, 250 mM and 10 mM respectively. The buffer was sterilized by filtration through a pre-rinsed 0.2 µm Nalgene filter. It was stored frozen at -20°C until used.

Preparation of competent culture of *E. coli*

Sterile techniques were required throughout the preparation of the competent culture. A single colony was picked from LB agar plate streaked with TOP 10 *E. coli* provided in His-Patch ThioFusion™ Expression System (Invitrogen™) and incubated for 17 h and transferred to 25 ml SOB medium. It was incubated at 37°C with 250 rpm shaking for 6 h. 0.8 ml of the start culture was used to inoculate 50ml fresh SOB medium, then it was incubated at 18°C with moderate shaking for 16 h. When the OD_{600} reached 0.55, the culture flask was transferred to ice-water bath for 10 min. The cooled culture was poured into a pre-chilled sterile 50 ml centrifuge tube and the cells were harvested at 13000 rpm for 10 min at 4°C in a Beckman F2402H rotor. The medium was removed and the cells were resuspended in 16 ml of sterilized ice-cold Inoue transformation buffer. The cells were harvested again at 13000 rpm for 10 min at 4°C in a Beckman F2402H rotor and resuspended in 4 ml of sterilized ice- old Inoue transformation buffer. 1.5 ml of DMSO was mixed with the cell suspension and keeping in ice for 10 min. The cell suspension was quickly aliquot in 50 µl into pre-chilled, sterile 1.5 ml microfuge tubes kept on ice. The tubes were then stored at –80°C until needed.

Construction of Recombinant 110 bpThioHis-B

The 110-kDa polypeptide antigen gene of *O. Tsutsugamushi* (Karp strain), open reading frame-B with respective to nucleotide positions 1990 – 3209, 1.22 kb DNA fragment, was amplified by PCR and the amplified products were cloned into expression plasmid (pThioHis B) of His-Patch ThioFusion™ Expression System, purchased from Invitrogen™ Life Technologies, to generate 110 bpThioHis-B construct. The strategy for cloning and construction of recombinant plasmid was showed in Fig 3.

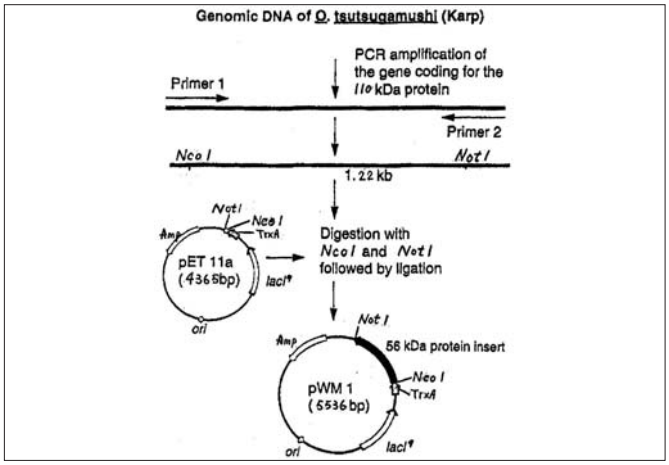


Figure 3. *Strategy of cloning and construction of 110 bpThioHis-B*

Polymerase Chain Reaction

Primer pair [1990Foward (5'-CTCTAATAACCATGGTGATTG CTGGAGATG AT-3') and 3209Reverse (5'-CTGGCGGCCG CTTTGCTAATTATTGCGAGGAG T-3')] was designed by using the nucleotide sequence of the open reading frame-B of the 110-kDa antigen gene from Karp strain of *O. tsutsugamushi.* The restriction sites for Nco I and Not I are underlined, and the new initiation codon ATG and reverse complement of the new stop codon CTA are shown.

PCR amplification was carried out in 50 µl reaction mixture. The mixture composed of 0.2 mM (each) deoxynucleoside triphosphate, 1X PCR buffer (200 mM Tris-HCl, pH 8.8 at 25°C; 100 mM (NH4)2 SO4; 100 mM KCl; 1% Triton X-100; 1mg/ml BSA; 20 mM MgSO4), 1 µM (forward and reverse) primer, 2.5 unit of Pfu DNA polymerase (Fermentas) and 0.1 µg template DNA. A control was prepared in which does not consist of template DNA. Thermocycler (Eppendorf) was used in the experiment. The PCR was started with 5 min at 94°C

and followed by 35 cycles, with 1 cycle consisting of 2 min at 95°C, 1 min at 60°C, and 3 min at 72°C. A final step of 5 min at 72°C was added to the last cycle. The three set PCR products were purified together with the StrataPrep PCR Purification Kit (Stratagene).

Propagation of pThioHis B vector

pThioHis B vector was propagated in the competent *E. coli* (TOP 10 strain). 100 ng of pThioHis B was gently mixed with 50 µl of competent *E. coli* in microcentrifuge tube. The tube was incubating on ice for 30 minutes. It was subjected to heat shock for 90 seconds in 42°C water bath and then placed on ice for 5 min. 250 ul of SOC medium was added to the tube and the tube was incubated at 37°C with moderate shaking for one hour. 10 µl of the cell suspension was spread on LB agar plate containing 100 µg/ml ampicillin. The plate was incubated 17 h in a 37°C incubator. A single colony of transformant was picked from the plate and transferred to 5 ml LB medium containing 100 µg/ml ampicillin. The inoculated medium was incubated in 37°C incubator with 200 rpm shaking for 16 h. Cell transformed with pThioHis B was harvested from the overnight culture and lysed for plasmid isolation using Concert Rapid Plasmid Miniprep System (Gibco-BRL Life Technologies™).

Restriction Enzyme Digestion and Purification

The washed PCR product and pThioHis B vector were digested by Nco I and Not I restriction endonuclease (BioLabs). Double digestion with Nco I and Not I was performed on the 50 µl washed PCR product and 30 µl pThioHis B to generate cohesive ends of Nco I and Not I. 20 units of Nco I and Not I were used in each double digestion reaction. NEBuffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl2, 1 mM dithiothreitol, pH 7.9 at 25°C) was the common buffer for the reactions that both enzymes would have 100% activity. The buffer was diluted to final concentration 1X in final reaction volumes. The digestion reactions were supplemented with BSA to final concentration of 100 µg/ml and incubated at 37°C for 16 h. The double digested PCR product and pThioHis B were run on 1% agarose gel for one hour at 95 V and then subjected to gel purification using the Concert Rapid Gel Extraction System (GibcoBRL Life Technologies™). The DNA concentration of each tube was determined.

Ligation and Transformation

Ligation reaction was performed by T4 DNA ligase (BioLabs). Three sets of vector-insert ligation reaction, one set of vector self-ligation reaction, and one set control containing only sterile distilled water were performed. Each set of reaction was in 20 µl final volume. 400 units of T4 DNA ligase were used in each reaction set except the control. Ligations were performed in final concentration 1X T4 DNA ligase buffer. In the three sets of vector-insert ligation, three vectors to insert molar ratio have been tried. They are 1:4, 1:10, and 1:20. 30 ng of vector and 32 ng, 80 ng, and 160 ng of insert were used in the 1:4, 1:10, and 1:20 ligation, respectively. The five reaction tubes were placed in 16°C for 18 h.

After ligation took place, the five reaction mixtures were used to transform competent *E. coli*, Top 10 strain, separately. They were gently mixed with 50 µl of competent *E. coli* in each microtube respectively. The tubes were incubating on ice for 30 min. They were subjected to heat shock for 90 s in 42°C water bath and then placed on ice for 5 min. 300 µl of SOC medium was added to each tube and the tubes were incubated at 37°C with moderate shaking for one hour. The five cell suspensions were spread on LB agar plates containing 100 µg/ml ampicillin respectively. The plates were incubated in a 37°C incubator for 17 h.

Restriction Analysis of Recombinant Clones

Each colony of ampicillin-resistance transformant was picked from the vector-insert ligation plates and propagated in 5 ml LB medium containing 100 µg/ml ampicillin at 37°C incubation under 200 rpm shaking for 16 h. Cells was harvested from the overnight cultures and lysed for plasmid isolation using Concert Rapid Plasmid Miniprep System (Gibco-BRL Life Technologies™).

The plasmid DNA isolated from the transformed cells was analyzed by restriction endonuclease (BioLabs). Double digestion with Nco I and Not I was performed on each plasmid DNA sample. 5 µl of each sample was mixed with 10 units of Nco I and Not I. NEBuffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl2, 1 mM dithiothreitol, pH 7.9 at 25°C) was diluted to final concentration 1X in final reaction volumes. The digestion reactions were supplemented with BSA to final concentration of 100 µg/ml and incubated at 37°C for 1 h. The digested samples were run on 1% agarose gel at 95 V for 1 h.

Reading Frame Confirmation by Nucleotide sequence Analysis

Based on the positive restriction analysis result, the recombinant DNA sample was submitted to Tech Dragon Limited for DNA sequencing in order to confirm the sequence, orientation and reading frame the inserted DNA fragment.

Keeping frozen stock of the recombinant clone

Glycerol stock of the desired clone was kept for safekeeping by mixing 0.85 ml of an overnight bacterial culture with 0.15 ml of sterile glycerol. It was kept in -80°C.

Growth curve of *E. coli* carrying recombinant DNA

Growth of *E. coli* (Top 10) carrying the recombinant DNA was studied. The clone of interest was streaked out on a LB plate with 100 µg/ml ampicillin and grown at 37°C for 17 h. A single colony from the plate was picked to inoculate 10 ml LB medium with 100 µg/ml ampicillin. It was then grown at 37°C, 200 rpm shaking incubator overnight. 1 ml of the overnight culture was used to inoculate 30 ml fresh LB medium with 100 µg/ml ampicillin in a 250 ml culture flask. The flask was incubated in 37°C, 200 rpm shaker water bath. The growth was monitored by spectrophotometer at 550 nm in 30 minutes time interval for the first six and last six time points, and in 15 minutes time interval for rest of the time points. Total 10.5 hours of the cell growth was monitored. 1 ml of culture was taken for OD reading at each time interval and fresh medium was used as blank. The OD_{550} reading of each time point were plotted to generate a growth curve.

Growth and induction of cells

A single colony of recombinant clone was picked to inoculate 5ml LB medium with 100 µg/ml ampicillin. It was grown overnight at 37°C, 200 rpm shaking incubator. 20 ml of fresh LB medium with 100 µg/ml ampicillin in a 250 ml culture flask was inoculated by 1 ml overnight culture and grown at 37°C with 200 rpm shaking. When OD_{550} = 0.5 was reached, 5 ml culture was removed from the culture flask and centrifuged at 14000 rpm to harvest the cell. The cell collected was labeled t= 0. This was time zero sample. After that, IPTG was added to the culture flask to final concentration 1 mM and the flask

was returned to the water bath. Other 5 ml of culture was removed for cell harvesting at 1.5 h interval after the addition of IPTG. So, there were cells from t= 0, t= 1.5, t= 3, and t= 4.5. The harvested cell pellets were kept at –20°C freezer. Two set of control were performed at the same pace along with the above procedure. The negative control was the untransformed Top 10 *E. coli* i.e. without plasmid while the positive control was Top 10 *E. coli* containing expression vector pThioHisB without DNA fragment insert. Totally, there were twelve cell samples in which four in a set.

Preparation of cell lysates and protein concentration determination

The twelve cell samples from growth and induction of cells was removed from -80°C and thawed quickly in 40-50°C water. Each cell pellet was resuspended in 200 µl cold (+4°C) buffer (20 mM Tris-HCl, pH 8, 2.5 mM EDTA, and 10 mM imidazole). The samples were subjected to sonication by hand-held sonicator with 6 mm microtip and sonicate one at a time for 10 s bursts. The lysates were centrifuged at 14000 rpm for five mins at 4°C. Supernatants were removed to fresh microcentrifuge tubes on ice. Concentration of protein of each supernatant sample was determined by Bradford protein assay, so that 20 µg of protein of each sample in 10 µl in volume was prepared for SDS-PAGE Gel analysis.

Protein Analysis by discontinuous SDS-PAGE

The twelve protein samples were analyzed by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Discontinuous buffer system and 12% separating gel was performed. 10 µl of 20 µg each protein sample were mixed with 10 µl 2X sample buffer. The mixtures were boiled at 100°C for 10 minutes. Total 20 µl of mixture of each sample was loaded into each well. Protein marker (Pharmacia) was loaded also. The gel was electrophoresed at 200 V for 1 h. Then, the gel was stained for 1 h, destained overnight, and then dried.

RESULTS

Construction of recombinant 110 bpThioHis-B

Double digestion of pThioHis-B and PCR product

The vector pThioHis-B and PCR product were double digested by the restriction endonuclease Nco I and Not I, and were purified by gel purification. 2 ul of the each purified product was run on the 1% agarose gel showed in Fig 4. The DNA concentration of each purified product was estimated. The concentration of vector pThioHis-B was 15 ng/µl and PCR product was 13 ng/µl.

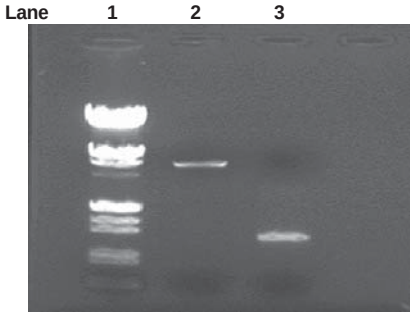


Figure 4. *Purified double digested vector pThioHis-B and PCR product. Lane 2 and 3 shows the vector pThioHis-B and PCR product with size of 4.4 kb and 1.2 kb respectively. Lane 1 is lambda DNA (digested EcoRI + HindIII) size marker (Fermentas).*

Transformation efficiency and Ligation efficiency

Transformation of competent E. coli Top 10 strain with intact vector pThioHis-B.

To determine number of transformation of *E. coli* Top 10 strain per µg of plasmid, competent *E. coli* Top 10 strain was transformed with 100 ng of intact pThioHis-B. Sixty-eight transformants were found on LB agar plate with ampicillin (100 µg/ml) when 10 µl of total 300 µl of transformed cells was plated. Transformation efficiency was 2.04 x 10^4 transformants per µg of plasmid, showed in Table 1.

Table 1. Calculation of transformation efficiency of competent Top 10 *E. coli* transformed with pThioHis

$$\frac{\#\text{ of colonies}}{100\text{ng pThioHis-B}} \times \frac{10^3\text{ ng}}{\mu\text{g}} \times \frac{300\mu\text{l of transformed cells}}{\text{X µl plated}} = \frac{\#\text{ of transformants}}{\mu\text{g of pThioHis-B}}$$

$$\text{Transformation Efficiency} = \frac{68}{100\text{ng}} \times \frac{10^3\text{ ng}}{\mu\text{g}} \times \frac{300\text{ul}}{10\mu\text{l}} = \frac{2.04 \times 10^4\text{ transformants}}{\mu\text{g of pThioHis-B}}$$

Transformation of competent E. coli Top 10 strain with ligation product

Ligation reactions: one control containing only sterile distil water, one set of vector self-ligation reaction excluding insert DNA, and three sets of vector-insert ligation reaction with molar ratio of vector to insert 1:4, 1:10, and 1:20, were performed. Each of the ligation transformed the competent *E. coli* Top 10 strain. The transformed cells were plated on five LB agar plates contain ampicillin (100 µg/ml) respectively and the results are showed in Table 2. One colony-forming unit was found on the self-ligation plate and the 1:20 plate. Transformation efficiency determined from the 1:20 plate was 33.3 transformants per µg of plasmid, showed in Table 3.

Table 2. Number of transformant formed in ligation with different molar ratio of vector to insert

	Control	Self-ligation	1:4	1:10	1:20
Description	Water only	No insert	Molar Ratio of Vector to Insert 1:4	Molar Ratio of Vector to Insert 1:10	Molar Ratio of Vector to Insert 1:20
Volume plated (µl)	300	300	300	300	300
Number of colonies (cfu)	0	1	0	0	1

Table 3. Calculation of transformation efficiency of competent Top 10 *E. coli* transformed with ligation product (vector to insert ratio 1:20).

$$\frac{\#\text{ of colonies}}{\text{30ng linearized pThioHis-B}} \times \frac{10^3\text{ ng}}{\mu\text{g}} \times \frac{300\mu\text{l of transformed cells}}{\text{X µl plated}} = \frac{\#\text{ of transformants}}{\mu\text{g of linearized pThioHis-B}}$$

$$\text{Transformation Efficiency} = \frac{1}{30\text{ng}} \times \frac{10^3\text{ ng}}{\mu\text{g}} \times \frac{300\mu\text{l}}{300\mu\text{l}} = \frac{33.3\text{ transformants}}{\text{ug of linearized pThioHis-B}}$$

Ligation efficiency

Ligation efficiency between vector and insert was calculated by comparing the transformation efficiency of competent cells with intact pThioHis-B and with vector-insert ligation product (Table 4). The ligation efficiency was 0.16% as shown in calculation.

Table 4. Calculation of Ligation efficiency

$$\text{Ligation Efficiency} = \frac{\text{transformation efficiency with intact pThioHis-B}}{\text{transformation efficiency with vector-insert ligation product}} \times 100\%$$

$$= (33.3 / 2.04 \times 10^4) \times 100\% = 0.16\%$$

Restriction analysis of the 110 bpThioHis-B construct

Ampicillin-resistance transformant was picked from the vector-insert ligation plate (1:20) and propagated for plasmid isolation. The plasmid DNA isolated from the transformants was digested by restriction endonuclease Nco I and Not I. The digested DNA was run on 1% agarose gel (Fig 5). Lane 1 was plasmid DNA from vector-insert ligation plate (1:20). In lane 1, there were two bands with size 4.4 kb and 1.2 kb. This indicated 1.2 kb insert have been ligated to 4.4 kb vector and showed the colony on 1:20-plate contains recombinant construct.

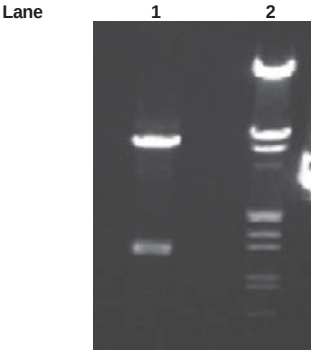


Figure 5. Result of double digestion of the isolated plasmids with restriction endonucleases Nco I and Not I. Lane 1: plasmid extracted from colony on ligation plate (1:20). Lane 2: lambda DNA (digested EcoRI + HindIII) size marker (Fermentas).

Reading Frame Confirmation by DNA sequencing

Upon affirmative confirmation of ampicillin-resistant transformants by restriction mapping, with a view to ensuring the gene of interested had been harbored in the right orientation and reading frame, 1 µg of purified recombinant constructs was submitted to DNA sequencing. In addition, primer GGCATCCCTGGTATCCCG targeted at about 100 base pairs upstream from the cloning site of the expression vector was designed to facilitate the sequencing. Subsequently, the sequenced nucleotides were aligned versus expression vector sequences by software ClustalX (Version 1.8) and GeneDoc (Version 2.0) and the alignment result is shown in Fig 6. The alignment clearly indicated that the gene of interested had been precisely cloned at the position of Nco I restriction site without shift of reading frame and change of orientation. Having known this, the right recombinant clone containing the desired gene was ready to overexpress the protein.

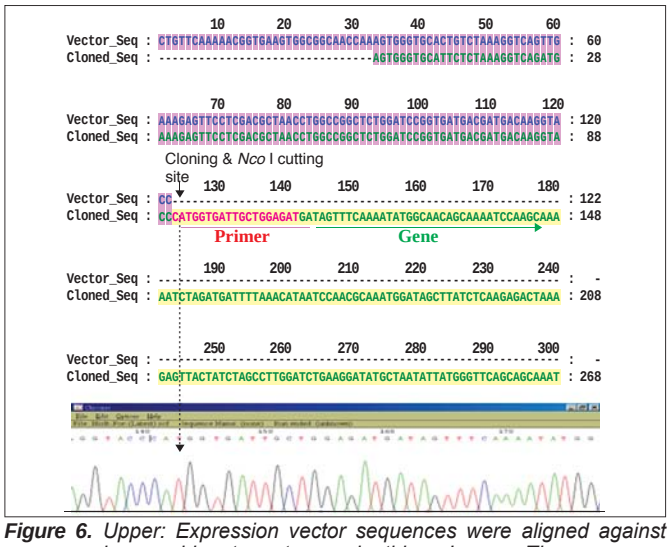


Figure 6. *Upper: Expression vector sequences were aligned against sequenced recombinant vector nucleotides. Lower: The sequence chromatography of the sequenced recombinant vector. As shown from above and indicated by dotted arrow line, the gene of interested were cloned at the position of Nco I restriction site without shift of reading frame and change of orientation.*

Growth curve of *E. coli* carrying recombinant DNA

The growth of *E. coli* carried the recombinant DNA was studied and the result was shown in Fig 7. Its growth was monitored by spectrophotometer at 550 nm from initial O.D 0.04 until 10.5 h growth was observed. The growth curve was generated.

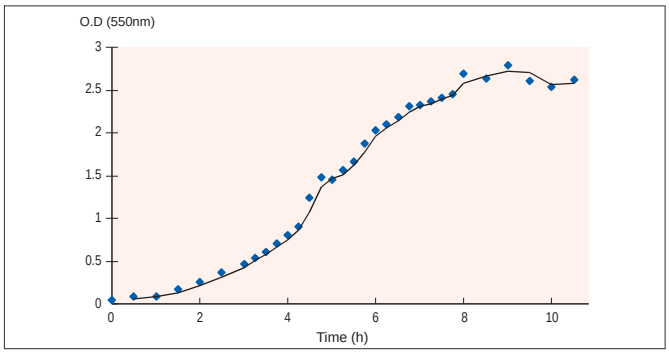


Figure 7. *Growth curve of TOP 10 E. coli carrying recombinant construct.*

Protein Analysis by SDS-PAGE Gel

The twelve protein samples from different time points were analyzed by discontinuous SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). 12% separating gel was used. Protein separation by SDS-PAGE was used to check for the presence, approximate molecular weights and relative abundance of the target proteins in the samples.

There were three sets of sample, cell lysates of untransformed Top 10 *E. coli*, cell lysates of Top 10 *E. coli* transformed with expression plasmid pThioHis-B, and cell lysate of Top 10 *E. coli* transformed with recombinant construct 110 bpThioHis-B, and each set consisted of four time points: t= 0, t= 1.5, t= 3, t= 4.5, in which cells in t= 0 was uninduced by IPTG while others were grown in 1 mM IPTG.

From the gel shown in Fig 8, it was found that there was expressed protein sized 61 kDa (estimated from standard protein marker) in the set of cell lysate of Top 10 *E. coli* transformed with recombinant construct 110 bpThioHis-B except the uninduced t= 0 sample. The expression increased as t increase. For cell lysates of Top 10 *E. coli* transformed with expression plasmid pThioHis-B, there was expression of the HP thioredoxin and multiple cloning site of known size (16 kDa), in lane 10 and 11. While the negative control, cell lysates of untransformed Top 10 *E. coli*, showed no expression in the four time points even they were induced by IPTG.

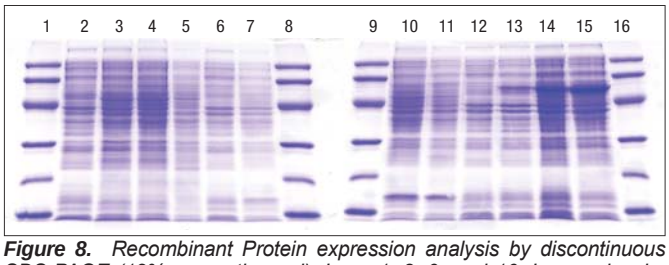


Figure 8. *Recombinant Protein expression analysis by discontinuous SDS-PAGE (12% separating gel). Lane 1, 8, 9, and 16: Low molecular weight protein marker (Pharmacia) sized (top) 94- (phosphorylase b), 67- (bovine serum albumin), 43- (ovalbumin), 30- (carbonic anhydrase), 20.1- (soybean trysin inhibitor), 14.4- (α-lactalbumin) kDa. Lane 2, 3, 4, and 5 were cell lysates of Top 10 E. coli without transformed and t= 0, t= 1.5, t= 3, t= 4.5 were respectively in lane 2 to 5. Lane 6, 7, 10, and 11 were cell lysates of Top 10 E. coli transformed with expression plasmid pThioHis-B and t= 0, t= 1.5, t= 3, t= 4.5 were respectively on lane 6, 7, 10, 11. Lane 12, 13, 14, and 15 were cell lysates t= 0, t= 1.5, t= 3, t= 4.5 respectively of Top 10 E. coli transformed with construct 110 bpThioHis-B.*

DISCUSSION

The expression of heterologous genes in bacteria is the simplest and most inexpensive means available for obtaining large amounts of desired proteins for research, clinical and industrial purposes. *E. coli* is the most widely used host for the production of recombinant proteins because of its well-characterized system, and amenability, both genetic and physiological. Due to availability of a various strong promoters and techniques, the efficient production of recombinant proteins in *E. coli* has become a routine matter. Furthermore, *E. coli* can readily be grown to high cell density with simple nutrients and can produce cloned proteins at high level (10-20% of total cell protein). This has led to the development of inexpensive high-yield fermentation processed for the production of therapeutic and industrial proteins on a large scale. Although *E. coli* is not a secretory bacterium, it can excrete proteins to its cell surface[23,24] and into the culture medium[25-27] if the right vector systems are used.

However, it had become apparent that *E. coli* could not be used to produce some eukaryotic proteins particularly those requiring post-translational modification for biological activity. These modifications include glycosylation, disulfide bridge formation, phosphorylation, proteolytic cleavage of precursor molecules and macromolecular assembly. In addition, many proteins expressed in *E. coli* were accumulated intracellular in the form of insoluble, inactive inclusion bodies, from which biological activity must be recovered by complicated and costly denaturation and refolding process.[28, 29] Furthermore, there is possibility of bacteriophage infection and contamination of products with endotoxins and pyrogens. This can be overcome in plant design and during product purification.[30]

E. coli expression systems are well suited for the expression of small proteins (<50kDa).[31] The demonstrated applications of *E. coli* system are therefore in synthesis of biologically active peptides such as alpha-interferon, human growth hormone, enzyme aspartase and other cloned bacterial products: cellulase (bacillus, cellulomonas sp.)[28, 32]

The choice of an expression system for the high-level production of recombinant proteins depends on many factors. These include cell growth characteristics, expression levels, and biological activity of the target protein. In addition, the selection of a particular expression system requires a cost down in terms of process, design, and other economic consideration.

E. coli Expression System

E. coli, gram negative bacteria, contains two membranes (inner and outer membrane) and can be divided three compartments by these two membranes: cytoplasmic, periplasmic, and extracellular space. The production of recombinant proteins are targeted to one of these three compartments and the mode of gene expression affects the location of the protein produced.

Most of the *E. coli* expression vectors used for high-level protein production contains strong regulated promoters that are based on the lac operon. The control region and N-terminal coding sequence of an *E. coli* gene is usually ligated to the coding sequence of a foreign gene. RNA polymerase recognizes the promoter as native and transcribes the gene. Because of many types of non-bacterial proteins are growth-inhibitory, or even toxic, when overexpressed in *E. coli*, protein expression is usually inhibited in these systems by lac repressor protein during culture scale-up. Protein expression can be reactivated by inducer IPTG. Also, *E. coli* expression vector are usually designed to produce fusion proteins that contain affinity "tags" at the amino or carboxyl terminus of the expressed protein so as to prepare homogeneous protein efficiently. Polyhistidine tag is an example that binds to Ni^{2}+ affinity column.[33, 34]

His-Patch ThioFusion expression system

In this project, open reading frame-B of 110-kDa polypeptide antigen gene, with respective to nucleotide positions 1990 – 3209 (1220 bp), of *O. tsutsugamushi* (Karp strain), was amplified by PCR, and the DNA fragment was cloned into expression plasmid (pThioHis-B) of His-Patch ThioFusion™ Expression System (Invitrogen™).

The His-Patch ThioFusion Expression System allows expression and purification of soluble heterologous proteins from *E. coli*. Foreign genes inserted into the multiple cloning site of the expression vector, pThioHis, are expressed as fusions to a modified version of the *E. coli* protein thioredoxin (trxA),[35, 36] Thioredoxin fusions not only express at high levels, the thioredoxin moiety appears to confer solubility to formerly insoluble heterologous proteins.[36, 37]

The 11.7 kDa thioredoxin protein is found in yeasts, plants, and mammals, as well as in bacteria. It was originally isolated from *E. coli* as a hydrogen donor for ribonuclease reductase[36]. When overexpressed in *E. coli*, thioredoxin is able to accumulate to approximately 40% of the total cellular protein. The thioredoxin gene in pThioHis has been mutated to create Histidine Patch thioredoxin, which bears a metal-binding domain in the thioredoxin protein. This histidine patch was shown to have high affinity for divalent cations. His Patch thioredoxin fusion can therefore be purified on metal-chelating resins.

High level expression of HP-thioredoxin fusion proteins is driven by the trc (*trp-lac* hybrid) promoter. The *trc* promoter contains the –35 region of the *trp* promoter together with the –10 region of the *lac* promoter. To regulate expression from the *trc* promoter, the gene encoding and overproducing Lac repressor ($lacI^{q}$) is provided in the pThioHis vector. Lac repressor occupies the lac operator to prevent initiating transcription by RNA polymerase. Isopropyl-β-D-thiogalactopyranoside (IPTG) is a de-repressor binding to Lac repressor and releasing it from Lac operator so as to induce expression of fusion protein. Translation is enhanced by the bacteriophage T7 gene 10 ribosome binding site that provides a high efficient initiation site for the translation of HP-thioredoxin fusions. Desired DNA is inserted into multiple cloning site, consisting of a number of restriction enzyme sites, allowing translational fusions to the 3' end of the modified *trx*A gene (HP-thioredoxin). *E. coli asp*A transcription terminator is positioned after multiple cloning site, ensuring efficient transcription termination of fusion protein.

An enterokinase cleavage site is engineered into pThioHis in a 30 bp spacer between the modified trxA gene and the multiple cloning site. Enterokinase recognizes the amino acid sequence Asp-Asp-Asp-Asp-Lys and hydrolyze the peptide bond between lysine and the next amino acid. This allows release of desired protein from HP-thioredoxin.

Also, the pThioHis expression plasmid carries pUC origin and ampicillin resistance open reading frame. The pUC origin allows the plasmid to maintain and replicate in *E. coli*, while the latter is a genetic marker for selection of *E. coli* containing the plasmid that the *E. coli* can survive in the presence of ampicillin.

TOP 10 *E. coli* is the host system for expression of heterologous protein. Like other type of *E. coli* host for recombinant DNA cloning experiment, it has mutations in the endogenous restriction modification system (*hsd*RMS$^-$), homologous DNA recombinant function (*rec*A$^-$) and endonuclease I activity (*end*A$^-$). The restriction modification system allows *E. coli* to resist foreign DNA that unmethylated DNA is degraded by site-specific restriction enzyme very shortly after entering the cell. This requires three proteins encoded by *hsd*R, *hsd*M, and *hsd*S genes that function together as a protein complex to cleave and to methylate DNA. So mutation of the genes permits the maintenance of foreign plasmid. The *rec*A gene encodes a well-characterized recombination protein, which would cause rearrangement or deletion of insert DNA. Mutation of *rec*A gene can ensure the integrity of introduced plasmid. The *end*A gene encodes DNA specific endonuclease I. Loss of this endonuclease greatly increase plasmid DNA yields and improves the quality of miniprep DNA.

Construction of Recombinant 110-bpThioHis-B & Protein Expression

Recombinant DNA method allows easy production of antigen in large amounts. The recombinant protein antigen offers a considerable advantage over the antigens derived directly from *O. tsustugamushi*, which requires cultivation and purification of *O. tsutsugamushi*. Although truncated recombinant variable 56-kDa protein antigen of *O. tsustugamushi* from Karp strain have been expressed and refolded for use in enzyme linked immunosorbent assays,[18] attempts to express the 110 kDa antigen has not been reported. Our work is attempting on construction of recombinant 110 kDa antigen. The recombinant protein can be applied in production of recombinant vaccine involving combination recombinant polypeptide antigens. As *O. tsutsugamushi* exhibits a variety of serotypes that limits the use of whole-cell-antigen vaccine to trigger full immunoprotection against difference strains.[21] In vaccine development, the variable 56 kDa antigen is the major candidate as it is the immunodominant antigen in scrub typhus infections.[18] It induced immunity against *O. tsutsugamushi* in animal models.[21, 38] However, full protection with 56-kDa immunization has not been achieved in outbred mice. So the vaccine development is heading to multivalent vaccine with a limited set of antigens. The recombinant 110 kDa protein may be a candidate for the multivalent vaccine based on that patient sera recognize this protein.[39]

The protein coding sequence for the 110 kDa antigen Karp strain was amplified by polymerase chain reaction (PCR). PCR is a direct enzymatic amplification process of a target DNA sequence to produce more than 10 million copies from only a few molecules. The use of a thermostable polymerase allows the dissociation of newly formed complimentary DNA and subsequent annealing or hybridization of primers to the target sequence with minimal loss of enzymatic activity. PCR requires knowing small amount nucleotide sequence at each end of the region to be amplified. Oligonucleotides, normally 15 – 30 bp, complementary to those sequences are synthesized used as primer for enzymatic amplification. PCR is by means of alternation of temperature in three steps: denaturation of template (dissociation of complimentary DNA), primer annealing (hybridization of oligonucleotides to target sequence), and elongation (enzymatic addition of nucleotides in 5' to 3' direction complementary to template). As the repetitive nature of the process, PCR machine is designed as automatical thermal cycler. *Pfu* DNA polymerase was used in PCR carried out in this project. This enzyme has proofing ability that ensures production of correct sequence, so that correct amino acid sequence could be resulted. There are a number of variable factors in PCR. The primer concentration should not be too high that encourage nonspecific binding and formation of primer dimer. Also, self-complementary between primers should be avoided. The melting temperature difference between primer pair should be within 4 to 6°C and the GC content of the primer should be 40-60%. The concentration of each dNTP in reaction should be the same so that accurate incorporation takes place, as if one dNTP is at a higher concentration it will be preferentially incorporated for this reason. For $MgSO_4$ concentration, too few Mg^{2+} ions result in a low yield of PCR product, too many increase the yield of non-specific products. The temperatures, duration, of each steps, and number of cycles for PCR are also variable factors; they depend on different kinds of amplification and the enzyme used.

Owing to restriction sites were incorporated to the primers, the PCR product would bear restriction site at each end. Ligation of DNA fragment of interest to expression vector was done by joining covalently of compatible sticky ends resulting from digestion by restriction endonuclease. Ligation was done with the T4 DNA ligase, which requires Mg^{2+} and ATP as cofactors. The reaction is usually carried out in 16°C. There are Nco I and Not I restriction sequence on the 5' end and 3'end respectively of the DNA fragment. Also, there are Nco I and Not I site on the 5' side and 3'side respectively of multiple cloning site on vector pThioHis-B. Digesting with Nco I and Not I, sticky ends of Nco I and Not I can be generated. After ligation, the DNA fragment can be inserted in frame into the cloning site of vector so as to produce desired protein translational fusions to the 3' end of the HP-thioredoxin gene. As the vector was cut by two restriction enzymes, this can minimize the possibility of rejoining of the vector without inserting the piece of DNA of interest owing to the inability of joining of non-compatible sticky ends during ligation.

From the result of ligation and transformation, it was found that the ligation efficiency was very low (0.16%). It was calculated by comparing the transformation efficiency (number of transformants per µg of pThioHis-B) of competent cells with intact pThioHis-B and with vector-insert ligation product. Also, it was found that the generation of recombinant clone occurred more likely when molar ratio of vector to insert decreased. Until the molar ratio of vector to insert decreased to 1:20, there was one recombinant clone. Furthermore, complete digestion of vector and insert with Nco I and Not I, that is both of them bears Nco I and Not I sticky end, would be one criteria to enhance the formation of recombinants and screening for the recombinants. From the result of self-ligation plate, there was only one colony. This showed almost all of the vector was digested by the two enzymes. For the control plate, no colony was found. It confirmed that the water used in all ligation reactions was sterile without contamination of plasmid.

After restriction analysis of the recombinant plasmid, DNA sequencing allows further confirmation of orientation and reading frame of the insert DNA. In addition, primer targeted at about 100 base pairs upstream from the cloning site of the expression vector was designed to facilitate the sequencing.

The identified recombinant construct was sequenced so as to confirm the reading frame not being shifted that correct polypeptide can be produced.

In the study of growth of *E. coli* carried the recombinant DNA in 10.5 h by O.D measurement, a typical growth curve was observed. The first 2 h of growth was the lag phase. From 2 to 8 h, cells grew exponentially. While from 8 to 10.5 h, it was the stationary phase in which no new growth is apparent. Having the general idea of the growth of cell, we can understand when the cells enters its exponential phase so as to induce expression of our target gene and estimate the time required for attaining certain O.D.

Analysis of protein expression by discontinuous SDS-PAGE

SDS-PAGE stands for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. SDS is an anionic detergent which binds strongly to protein at a constant ratio such that all the SDS-treated proteins are negatively charged with the same mass to charge ratio. The negative charges on SDS destroy most of the complex (secondary and tertiary) structure of proteins, giving them linearity, and are strongly attracted toward an anode (positively-charged electrode) in an electric field. The polyacrylamide gel is a cross-linked matrix that functions as a sieve. The negatively charged protein molecules are pulled to the positive end by the current, but they encounter resistance from this polyacrylamide mesh. The smaller molecules are able to navigate the mesh faster than the larger one, so they make it further down the gel than the larger molecules. Because the charge-to-mass ratio is nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in molecular weight (MW) of polypeptides.

There are two types of buffer systems in electrophoresis, continuous and discontinuous. A continuous system has only a single separating gel and uses the same buffer in the tanks and the gel. In a discontinuous system, a low percentage of acrylamide non-restrictive large pore gel, called stacking gel, is layered on top of a separating gel with smaller pore size. Each gel is made with a different buffer, and the tank buffers are different from the gel buffers. The original pH in the two layers is also different. These, together with the difference in the mobile ions, are crucial for the stacking effect in which the protein is sandwiched and concentrated between the leading and the trailing ions. The resolution obtained in a discontinuous system is much greater than that obtained with a continuous system.

In this project, SDS-PAGE was used to check for the presence, approximate molecular weights and level of expression of recombinant proteins in the samples. The cell lysates of TOP 10 *E. coli* carried the recombinant construct 110 bpThioHis-B showed that the recombinant protein was expressed as fusion protein only after induction by IPTG. This showed that the expression vector is highly regulated. The size of the fusion protein estimated according protein marker on the gel was 61 kDa. This size was the sum of 110-kDa antigen polypeptide fragment (45 kDa) and HP thioredoxin (16 kDa). The gel indicated that level of expression steadily increase with the time after induction. In the period of 4.5-hour post induction, maximum protein expression was at 4.5 hours after induction (t= 4.5), so this is the optimum incubation time for protein expression when the protein is overexpressed. As only 4.5 h after induction was monitored, study beyond 4.5 h should be conducted for the comprehensive trend of expression level verse time of incubation. The untransformed cell sample was served as a negative control while the cell transformed with pThioHis-B was served as a positive control that showed only the expression of HP thioredoxin and multiple cloning site which is 16 kDa in size. Although the amount of protein adding to each well of the gel was determined, there were still uneven quantities of protein in the wells. Luckily, the expression bands were obvious that the trend and difference between expression time points could still be observed.

Suggestion for further investigation

After determining the optimum incubation time for expression, the growth of cell can be scale up to larger volume of cultivation so large quantity of interested protein could be produced. Since there are lots of reports indicating that plasmid may lose during scale up, a systemic study of physiology of the recombinant cell is desirable. Down-stream processing of the protein can be troublesome. Extraction of the protein from broken cells could be complicated. Fortunately a logical approach to setup the pilot scale up of purification could most likely be achievable on column which selectively adsorbs HP thioredoxin fusion protein.

CONCLUSION

The open reading frame B of 110 kDa antigen gene of *Orientia tsutsugamushi* Karp strain was amplified by PCR and successfully cloned into expression vector pThioHis-B to generate recombinant construct 110 bpThioHis-B. The recombinant DNA was found in ligation of 1:20 molar ratio of vector to insert. The ligation efficiency was 0.16%. DNA sequencing showed the DNA fragment was inserted in frame. The construct was expressed in TOP 10 *E. coli* host. The protein was expressed as fusion protein to HP-thioredoxin. Expression of protein was highly regulated and IPTG was inducer to trigger expression. The expressed fusion was 61 kDa in size in which 16 kDa was belonged to HP thioredoxin and 45 kDa was the antigen polypeptides. It was overexpressed in 4.5 h post IPTG induction.

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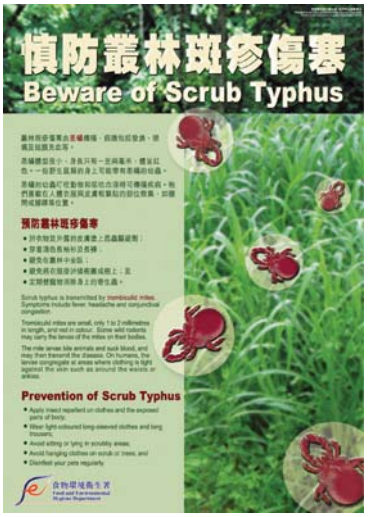

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References

1. Seong SY, Choi MS, Kim IS (2001). *Orientia tsutsugamushi* infection: overview and immune responses. ***Microbes Infect*** **3**: 11-21.
2. Keller CA, Hauptmann M, Kolbaum J, Gharaibeh M, Neumann M, et al. (2014). Dissemination of *Orientia tsutsugamushi* and inflammatory responses in a murine model of scrub typhus. ***PLoS Negl Trop Dis*** **8**: e3064.
3. Rosenberg R (1997). Drug-resistant scrub typhus: Paradigm and paradox. ***Parasitol Today*** **13**: 131-132.
4. Parola P, Paddock CD, Raoult D (2005). Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. ***Clin Microbiol Rev*** **18**: 719-756.
5. Moron CG, Popov VL, Feng HM, Wear D, Walker DH (2001) Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. ***Mod Pathol*** **14**: 752-759.
6. Service MW (2000). Medical entomology for students. New York: Cambridge University Press, Cambridge. pp. p.256-262.
7. Mansueto P, Vitale G, Cascio A, Seidita A, Pepe I, et al. (2012). New insight into immunity and immunopathology of Rickettsial diseases. ***Clin Dev Immunol*** **2012**: 967852.
8. Koo JE, Hong HJ, Dearth A, Kobayashi KS, Koh YS (2012). Intracellular invasion of *Orientia tsutsugamushi* activates inflammasome in asc-dependent manner. ***PLoS One*** **7**: e39042.
9. Rikihisa Y, Ito S (1979). Intracellular localization of *Rickettsia tsutsugamushi* in polymorphonuclear leukocytes. ***J Exp Med*** **150**: 703-708.
10. Lin PR, Tsai HP, Tsui PY, Weng MH, Kuo MD, et al. (2011). Genetic typing, based on the 56-kilodalton type-specific antigen gene, of *Orientia tsutsugamushi* strains isolated from chiggers collected from wild-caught rodents in Taiwan. ***Appl Environ Microbiol*** **77**: 3398-3405.
11. Moron CG, Popov VL, Feng H-M, Wear D, Walker DH (2001). Id`ntification of the Target Cells of *Orientia tsutsugamushi* in Human Cases of Scrub Typhus. ***Mod Pathol*** **14**: 752-759.
12. Nakayama K, Kurokawa K, Fukuhara M, Urakami H, Yamamoto S, et al. (2010). Genome comparison and phylogenetic analysis of *Orientia tsutsugamushi* strains. ***DNA Res*** **17**: 281-291.
13. Lin PR, Tsai HP, Weng MH, Lin HC, Chen KC, et al. (2014). Field assessment of *Orientia tsutsugamushi* infection in small mammals and its association with the occurrence of human scrub typhus in Taiwan. ***Acta Trop*** **131**: 117-123.
14. Amano K, Tamura A, Ohashi N, Urakami H, Kaya S, et al. (1987). Deficiency of peptidoglycan and lipopolysaccharide components in *Rickettsia tsutsugamushi*. ***Infect Immun*** **55**: 2290-2292.
15. Chen HW, Zhang Z, Huber E, Mutumanje E, Chao CC, et al. (2011). Kinetics and magnitude of antibody responses against the conserved 47-kilodalton antigen and the variable 56-kilodalton antigen in scrub typhus patients. ***Clin Vaccine Immunol*** **18**: 1021-1027.
16. Chen WJ, Niu DS, Zhang XY, Chen ML, Cui H, et al. (2003). Recombinant 56-kilodalton major outer membrane protein antigen of *Orientia tsutsugamushi* Shanxi and its antigenicity. ***Infect Immun*** **71**: 4772-4779.
17. Lu HY, Tsai KH, Yu SK, Cheng CH, Yang JS, et al. (2010). Phylogenetic analysis of 56-kDa type-specific antigen gene of *Orientia tsutsugamushi* isolates in Taiwan. ***Am J Trop Med Hyg*** **83**: 658-663.
18. Ching WM, Wang H, Eamsila C, Kelly DJ, Dasch GA (1998). Expression and refolding of truncated recombinant major outer membrane protein antigen (r56) of *Orientia tsutsugamushi* and its use in enzyme-linked immunosorbent assays. ***Clin Diagn Lab Immunol*** **5**: 519-526.
19. Park CS, Kim IC, Lee JB, Choi MS, Choi SB, et al. (1993). Analysis of antigenic characteristics of *Rickettsia tsutsugamushi* Boryong strain and antigenic heterogeneity of *Rickettsia tsutsugamushi* using monoclonal antibodies. ***J Korean Med Sci*** **8**: 319-324.
20. Oaks EV, Rice RM, Kelly DJ, Stover CK (1989). Antigenic and genetic relatedness of eight *Rickettsia tsutsugamushi* antigens. ***Infect Immun*** **57**: 3116-3122.
21. Seong SY, Huh MS, Jang WJ, Park SG, Kim JG, et al. (1997). Induction of homologous immune response to *Rickettsia tsutsugamushi* Boryong with a partial 56-kilodalton recombinant antigen fused with the maltose-binding protein MBP-Bor56. ***Infect Immun*** **65**: 1541-1545.
22. Valbuena G, Walker DH (2012). Approaches to vaccines against *Orientia tsutsugamushi*. ***Front Cell Infect Microbiol*** **2**: 170.
23. Yoon SH, Kim SK, Kim JF (2010). Secretory production of recombinant proteins in *Escherichia coli*. ***Recent Pat Biotechnol*** **4**: 23-29.
24. Chen N, Hong FL, Wang HH, Yuan QH, Ma WY, et al. (2012). Modified recombinant proteins can be exported via the Sec pathway in *Escherichia coli*. ***PLoS One*** **7**: 13.
25. Albiniak AM, Matos CF, Branston SD, Freedman RB, Keshavarz-Moore E, et al. (2013). High-level secretion of a recombinant protein to the culture medium with a *Bacillus subtilis* twin-arginine translocation system in *Escherichia coli*. ***FEBS J*** **280**: 3810-3821.
26. Takemori D, Yoshino K, Eba C, Nakano H, Iwasaki Y (2012). Extracellular production of phospholipase A2 from Streptomyces violaceoruber by recombinant *Escherichia coli*. ***Protein Expr Purif*** **81**: 145-150.
27. Meng X, Shang H, Zheng Y, Zhang Z (2013). Free fatty acid secretion by an engineered strain of *Escherichia coli*. ***Biotechnol Lett*** **35**: 2099-2103.
28. Kamionka M (2011) Engineering of therapeutic proteins production in *Escherichia coli*. ***Curr Pharm Biotechnol*** **12**: 268-274.
29. Ozgur A, Tutar Y (2013) Therapeutic proteins: A to Z. ***Protein Pept Lett*** **20**: 1365-1372.
30. Magalhaes PO, Lopes AM, Mazzola PG, Rangel-Yagui C, Penna TC, et al. (2007). Methods of endotoxin removal from biological preparations: ***a review. J Pharm Pharm Sci*** **10**: 388-404.
31. Dyson MR, Shadbolt SP, Vincent KJ, Perera RL, McCafferty J (2004). Production of soluble mammalian proteins in *Escherichia coli:* identification of protein features that correlate with successful expression. ***BMC Biotechnol*** **4**: 32.
32. Park SH, Pack MY (1986). Cloning and expression of a Bacillus cellulase gene in *Escherichia coli*. ***Enzyme and Microbial Technology*** **8**: 725-728.
33. Osadska M, Bonkova H, Krahulec J, Stuchlik S, Turna J (2014). Optimization of expression of untagged and histidine-tagged human recombinant thrombin precursors in *Escherichia coli*. ***Appl Microbiol Biotechnol*** **98**: 9259-9270.
34. Terpe K (2003). Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. ***Appl Microbiol Biotechnol*** **60**: 523-533.
35. Bayer ME (1968). Areas of adhesion between wall and membrane of *Escherichia coli*. ***J Gen Microbiol*** **53**: 395-404.
36. Holmgren A (1985). Thioredoxin. ***Annu Rev Biochem*** **54**: 237-271.
37. LaVallie ER, DiBlasio EA, Kovacic S, Grant KL, Schendel PF, et al. (1993) A thioredoxin gene fusion expression system that circumvents inclusion body formation in the *E. coli* cytoplasm. ***Biotechnology*** **11**: 187-193.
38. Seong SY, Kim HR, Huh MS, Park SG, Kang JS, et al. (1997a). Induction of neutralizing antibody in mice by immunization with recombinant 56 kDa protein of *Orientia tsutsugamushi*. ***Vaccine*** **15**: 1741-1747.
39. Ohashi N, Tamura A, Suto T (1988). Immunoblotting analysis of anti-rickettsial antibodies produced in patients of *Tsutsugamushi disease*. ***Microbiol Immunol*** **32**: 1085-1092.

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Chemical Constituents and Biological Activities of *Lobelia chinensis*

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Botanical Name: *Lobelia chinensis* Lour.
Plant Family: Campanulaceae
Pharmcopoeia Name: *Lobeliae Chinensis* Herba
Chinese Name: 半邊蓮 (Banbianlian)
Other Names: Chinese *Lobelia*, *Lobelia Chinensis* Herba, Aze Mushiro, Mizo Kakushi, Ji Jiesuo, Xi Micao, Ban Bianhua, Shui Xian Hua Cao, Lian Me Zai Cao
Part Used: Whole dried herb

ABSTRACT

***Lobelia chinensis* Lour is a species of commonly used herb in traditional Chinese medicine. It is listed in current Chinese Pharmacopoeia and conventionally utilized for cleansing body heat and toxins. Although this herb is belonged to the genus *Lobelia*, its chemical constituents and biological activities are different from *L. inflate*, which is an indigenous species in North America. The major bioactive constituents of *L. chinensis* are secondary metabolites belonged to piperidine alkaloids and flavonoids. These chemical components offer various health benefits, including anti-virus, anti-cancer and choleretic effects which have been confirmed and validated using modern pharmacological methods. In this article, some recent researches of the chemical constituents, biological activities and the medicinal applications of *L. chinensis* are reviewed.**

Keywords: *Lobelia chinensis; traditional Chinese medicine; chemical constituent; piperidine alkaloids; biological activities; anti-cancer*

INTRODUCTION

Lobelia *chinensis* Lour (Figure 1A), also known as "Chinese *Lobelia*" or "banbianlian" in Chinese, is a perennial plant of Campanulaceae family.[1] In the genus of *Lobelia*, there are around 350 species in the world. *L. chinensis* differs from *L. inflate*. The former species distributes mainly distributes in East Asia countries, such as China, Japan and Korea while the later is a native flora of North America. In China, more than 20 species of Lobelia have been identified but only 13 of them are used as herbal medicines.

L. chinensis was firstly mentioned in "Dian Nan Ben Cao", an old Chinese medicine book of Yunnan province. It was also recorded in the famous Chinese medicinal book "Ben Cao Gang Mu". It has been claimed effective for promoting urination, reducing edema, cleansing heat and toxicants. Previous studies on chemical constituents of this herb revealed a variety of piperidine alkaloids, flavonoids and lignans present in this plant.[2-4] As a widely grown herb in China, the whole dried plant has been used for the medicinal treatment of snake-biting, edema and diarrhea as indicated in the Chinese Pharmacopoeia.[5] Modern pharmacological investigations have indicated that *L. chinensis* exhibits various biological effects; it is choleretic, diuretic, anti-viral, anti-inflammatory, anti-proliferation of arterial smooth muscle cells, and so on.[6-8] Because of these many effects, this herb is a valuable herbal medicine. In this article, the chemical constituents and biological activities of *L. chinensis* are systematically reviewed.

DESCRIPTION AND IDENTIFICATION

L. chinensis, as illustrated in Figure 1A and 1B, is a small perpennial herb collected in summer. The herb is around 15-35 in length. After cleaning and drying under the sun, the herb was sold in bunchy as shown in Figure 1C. The rhizome of this herb is cylindrical, around 1-2 mm in diameter with pale brown or pale brownish-yellow on the surface. Its stem is long, thin and branching. The roots are slender, pale green and obviously multimodal; some of which are bearing fibrous. Leaves are normally alternate, sessile, mostly crumpled and greenish-brown, which are narrowly lanceolated around 0.8-3.2 cm long and 0.2-0.5 cm wide, after flattened. The pedicel is slender. The flower is small, solitary and axillary, with a tubular corolla at the base, which is five-lobed at the upper part and oblique on one side. The herb has slight odour, and tastes slightly sweet and pungent.

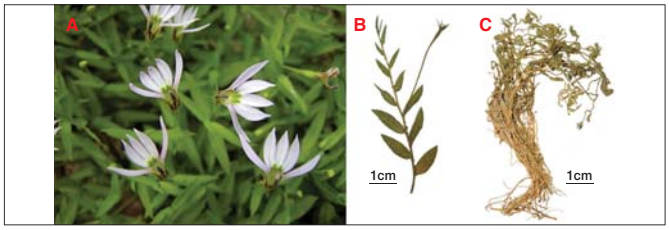
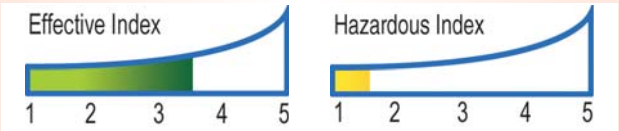


Figure 1 *Morphological features of Lobelia chinensis Lour. (A) Fresh plants; (B) Leaves and flower after drying; (C) Dried herbs.*



Contraindications

No obvious contraindications reported.

Undersirable Effects

Excessive oral consume may have abdominal pain and diarrhea. Other than these, no obvious toxicity reported.

Interaction with Conventional Drugs

No obvious adverse event reported.

MICROSCOPIC FEATURES

Root

As shown in Figure 2, the epidermis of this herb consists of one layer of cells, which is covered with cuticle. The cortex is broad. The parenchymatous cells are irregular and contain inulin and the aerenchyma is visible in the cortex. The endodermis is distinct with rectangular or square cells containing visible casparian dots. The vascular cylinder is small and the pericycle consists of one layer of parenchymatous cells. The laticiferous tubes are scattered in the phloem. Phloem bundles are arranged alternatively with xylem bundles. The centre usually shows a pith.

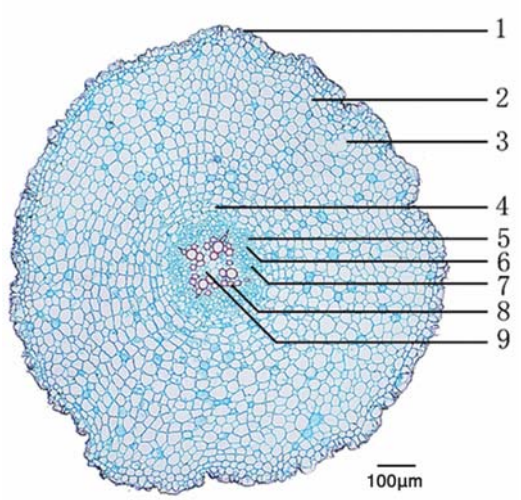


Figure 2 *Microscopic feature of transverse section of the root of Lobelia chinensis. 1 = Epidermis, 2 = Cortex, 3 = Aerenchyma, 4 = Endodermis, 5 = Pericycle, 6 = Phloem, 7 = Laticiferous tubes, 8 = Xylem, 9 = Pith*

Stem

Figure 3 shows the epidermis which consists of one layer of cells and covered with square or sub-square cuticle. The cortex is broad. The parenchymatous cells are polyhedral and sparsely arranged, and contain inulin. The aerenchyma is visible and clusters of calcium oxalate are occasionally found in the cortex. The endodermis is distinct, with rectangular or square cells. Casparian dots are visible. The vascular cylinder is small and the phloem is arranged in a ring with scattered laticiferous tubes. The xylem is radially arranged. The centre shows a pith.

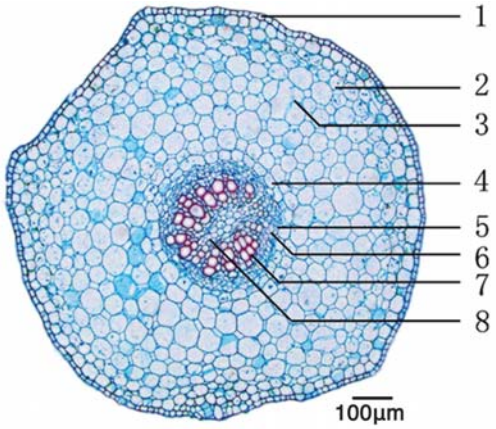


Figure 3 *Microscopic feature of transverse section of the stem of Lobelia chinensis. 1 = Epidermis, 2 = Cortex, 3 = Aerenchyma, 4 = Endodermis, 5 = Phloem, 6 = Laticiferous tubes, 7 = Xylem, 8 = Pith*

Leaf

As shown in Figure 4, the upper epidermis of a leaf of *L. chinensis* consists of one layer of sub-square cells, which are elongated tangentially and covered with cuticle. The palisade tissue consists of one layer of cells across the upper part of the midrib. The spongy tissue cells are sub-rounded while the vascular cylinder is small and collateral in type. The lower epidermis consists of one layer of sub-square cells, which is elongated tangentially and covered with cuticle. Unicellular non-glandular hair arises from the lower epidermis and is occasionally visible.

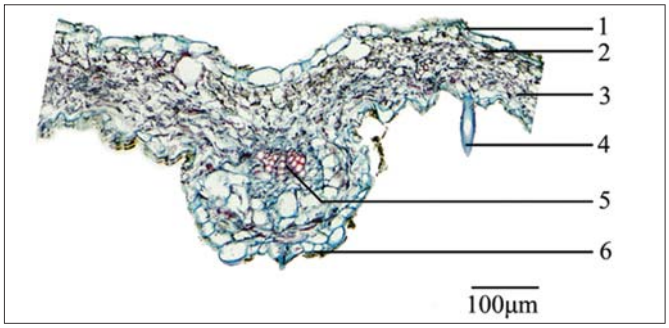


Figure 4 *Microscopic feature of transverse section of the leaf of Lobelia chinensis. 1 = Upper epidermis, 2 = Palisade tissue, 3 = Spongy tissue, 4 = Unicellular non-glandular hair, 5 = Vascular cylinder, 6 = Lower epidermis*

Powder

Pulverized powder of this herb appears in greyish greenish-yellow or brownish-yellow (Figure 5). Epidermal cells of the leaf have sinuous anticlinal walls. Epidermal cells of stem are irregularly rectangular in shape. The stomata are anomocytic with 3-7 subsidiary cells. The laticiferous tubes have alongside vessel, containing granular and oily contents. Parenchymatous cells, which contain inulin and are fan-shaped or irregularly shaped, are visible under a polarized microscope. Crystals of calcium oxalate are frequently found beside vessels, which are sometimes arranged in rows and polychromatic under the polarized microscope. Spiral vessels and reticulate vessels are visible and 6-67 µm in diameter. Non-glandular hairs are occasionally visible, and found in two types: one type is clavate shape, which contains 1-3 cells with slightly thicked walls, and another type is short conical shape, which is unicellular with warty walls in surface.

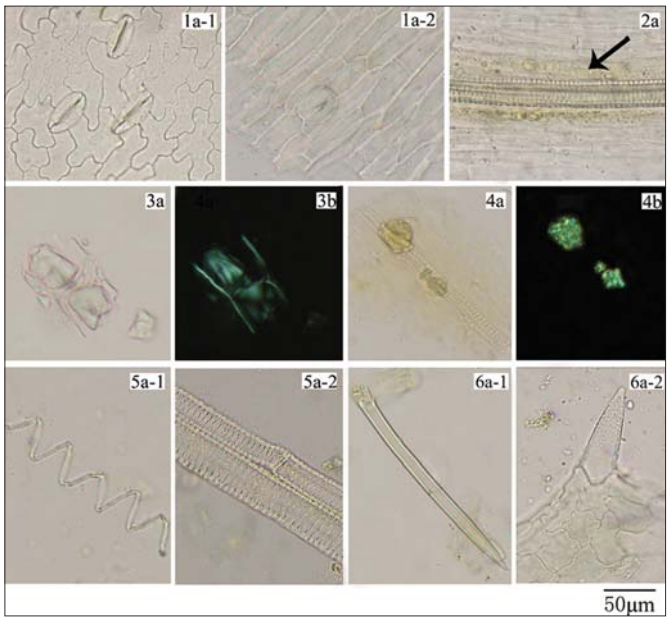


Figure 5 *Microscopic features of the powder of Lobelia chinensis. 1 = Epidermal cells and stomata (1a-1. leaf; 1a-2. stem), 2 = Laticiferous tube, 3 = Inulin, 4 = Crystals of calcium oxalate, 5 = Vessel (5a-1. Spiral vessel; 5a-2. Reticulate vessel), 6 = Non-glandular hair (6a-1. Clavate shape; 6a-2. Short conical shape). (a = Feature under the light microscope, b = Feature under the polarized microscope)*

CHEMICAL CONSTITUENTS

Along with the development of modern analysis techniques, more and more chemical constituents in *L. chinensis* have been successfully isolated and identified. Up till now, besides the primary metabolites glucides and amino acids, various kinds of secondary metabolites, such as alkaloids, flavonoids, lignans, cumarins, terpenes and polyacetylenes, have been detected as the major bioactive constituents of this herb.[4, 8]

Alkaloids

As the major and characteristical constituents of the genus *Lobelia*, various piperidine alkaloids, for example radicamines (A and B) (Figure 6), andrachcinidine (Figure 6) and lobechidines (A, B and C), have been isolated from *L. chinensis* by Kusano *et al* and Ren *et al*, respectively.[2, 9] The subsequent studies about the isolated piperidine alkaloids found these alkaloids could be inhibitors of α-glucosidase and showed potential anti-cancer activities. The total content of all alkaloids could reach 17.10 mg in one gram of the dried herbs.[10] However, *L. chinensis* is different from the other most studied *Lobelia* plant *L. inflata*, because there is no solid evidence to prove the existence of typical piperidine alkaloid lobeline in this species. Even though lobeline was usually considered to be the major ingredient of *L. chinensis* like *L. inflate* in many previous studies.[8, 11-12] Current research about the phytochemical profile of *L. chinensis* could only reach the conclusion that piperidine alkaloids are the major secondary metabolites in this herbal medicine.[9, 10]

In our research, comparing the high performance liquid chromatography (HPLC) chromatograms of lobeline standard sample to *L. chinensis*, the peak corresponding to lobeline in standard sample was not detected in *L. chinensis* as shown in Figure 7. Hence, we conclude that lobeline is absent in *L. chinensis* and it should not be used as a marker for its authentication or qualitative determination.

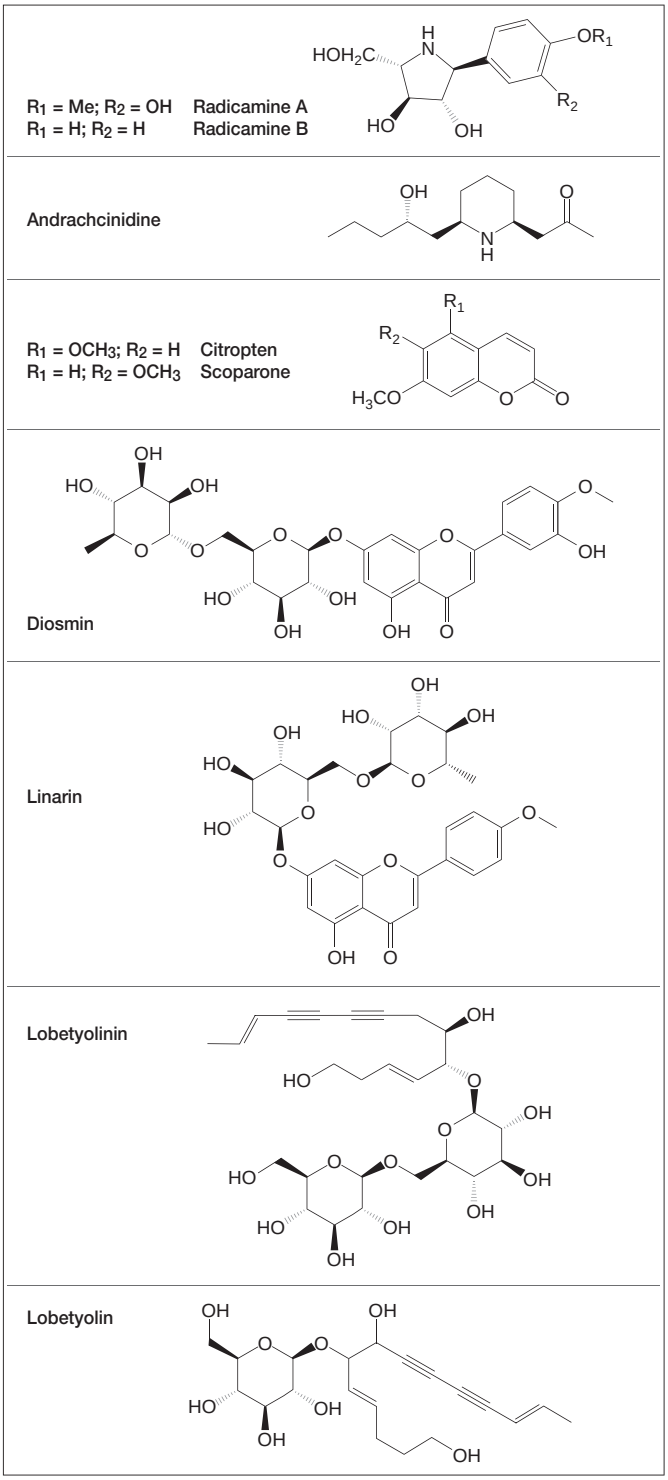


Figure 6 *Chemical structures of some compounds isolated from Lobelia chinensis.*

Flavonoids

Flavonoids are common secondary metabolites in many herbs. In *L. chinensis*, up to 4.7 $mg \cdot g^{-1}$ flavonoid glycosides could be obtained using ultrasonic wave-assisted semi-bionic extraction, and the antioxidant activities of these obtained flavonoid glycosides were evaluated by Pan *et al* also.[13] In the further study about the chemical constituents of *L. chinensis*, many kinds of flavonoids, such as apigenin, chrysoeriol, diosmetin, diosmin (Figure 6), hesperidin, linarin (Figure 6), luteolin and so on, were chromatographically isolated and identified with the assistance of multi-spectral data analysis.[14]

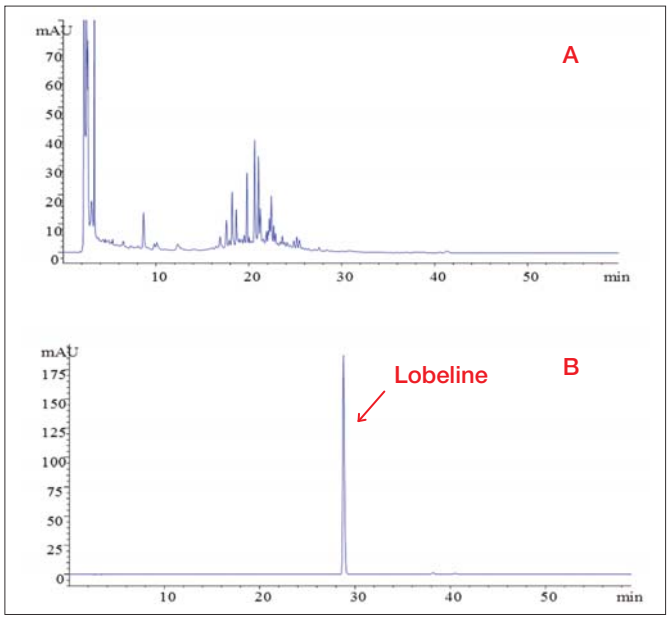


Figure 7 *HPLC chromatogram comparison between Lobelia chinensis (A) and lobeline standard sample (B).*

Lignans

Lignan are a kind of phenylpropanoids widely existed in plants. As the co-passengers of dietary fiber, lignans are especially abundant in many fiber-rich plants. In 2010, two pairs of enantiomeric neolignands and five common lignans were successfully isolated from the ethanol extract of *L.chinensis* by Ye *et al*.[3]

Coumarins

Coumarins are a group of phytochemicals with a vanilla like flavor. They belong to the family of phenylproanoids also and could be found in many plants. Two kinds of coumarins, citropten and scoparone (Figure 6), were isolated and identified from the whole plants of *L. chinensis* by Leu *et al*.[5] The anti-inflammatory bioactivity of scoparone by inhibiting the generation of superoxide anions was uncovered also.

Polyacetylenes

Polyacetylenes belong to a very special group of phytochemicals found in plants. As the typical polyacetylene constituents in the family of Campanulaceae, lobetyolin and lobetyolinin, were also found in *L. chinensis* and indicated potential markers for quality control.[4,15]

Other chemical constituents

Besides the phytochemicals summarized above, L-tyrosine, glucides, D-glucose, D-fructose, caffeic acid, *p*-coumaric acid, gallic acid, succinic acid, vanillic acid and benzoic acid and some other chemical constituents were also found in *L. chinensis* after extraction and isolation procedures.[3,4]

BIOLOGICAL ACTIVITIES

As a famous herbal medicine, *L. chinensis* has already been used to cure snake-biting, diarrhea, edema, inflammation, jaundice with damp-heat pathogen and eczema tetter in TCM for a long history. To make better use of this herbal medicine and reveal the relationship between the chemical constituents of *L. chinensis* and its bioactivities, the extracts and the isolated ingredients have been investigated using modern scientific pharmacological methods. Evidences accumulated in a wide range of bioactivities of *L. chinensis*, including anti-virus, anti-inflammatory, anti-endothelin-1, anti-cancer and choleretic activities.[4, 6-8, 16-20]

Anti-virus activity

Methanolic extract from *L. chinensis* exhibited a sustained protective effect against Herpes Simplex Virus Type I (HSV-1) through blocking HSV-1 replication in HeLa cells both *in vitro* and *in vivo*.[6] The half maximal inhibitory concentration (IC_{50}) for HSV-1 replication was calculated to be 139.2 $\mu g \cdot ml^{-1}$. In the plaque reduction assay, the ground skin samples from *L. chinensis* extract treated mice showed markedly decline in the HSV-1 titers and DNA levels, compared to control group. These results demonstrated that *L. chinensis* might be a potential alternative to treat acyclovir-resistant HSV-1 infection. The anti-virus activities of the crude extract, different solvents soluble compounds and pure isolated compounds were also examined by the screening of their inhibition of HSV-1 replication by Leu *et al* in 2011.[4] Finally, obvious anti-virus activities to HSV-1 of some tested samples from *L. chinensis* could be found.

Anti-inflammatory activity

Leu *et al* also screened selected fractions and pure compounds from chloroform soluble mixture of *L. chinensis* for their anti-inflammatory activities.[4] Scoparone, one of the active principles, significantly inhibited superoxide anion generation in a concentration-dependent manner with IC_{50} of 6.12 ± 1.79 μM, while, lobechine demonstrated as a moderate inhibitor of elastase release with IC_{50} of 25.01 ± 6.95 μM. The anti-inflammatory activities of different extracts from *L. chinensis* were also investigated using mouse abdominal cavity capillary permeability increase test, mouse auricle swelling test and mouse toe swelling test respectively.[16] The final results of these research reached a conclusion that *L. chinensis* could show obvious anti-inflammatory effect.

Anti-proliferation of arterial smooth muscle cells

Animal models have been used to demonstrate *Lobelia chinensis* as an antagonist of endothelin-1, which prevents the vascular smooth muscle cells from proliferation. Further research performed by Hu *et al* also showed an anti-proliferative effect on cultured human umbilical vascular smooth muscle cells and indicated that this effect was mediated by the reduce of increased [Ca^{2+}] rather than nonspecific cytotoxicity.[17]

Anti-cancer activity

In 2006, Zhang *et al* investigated the hepatoma cells apoptosis through the calcium pathway induced by *L. chinensis* and found this herbal medicine could induce human hepatoma cell $HepG_2$ apoptosis by significantly increasing the cytoplasmatic free Ca^{2+} level in $HepG_2$ (Figure 8).[18] In 2013, Yan *et al* used DMH (1,1-Dimethyl hydrazine)-induced rat colon carcinogenesis model to study the effects of *L. chinensis* on colon precancerous lesions.[19] They found that rats, which were orally administered with *L. chinensis* aqueous extract, showed dose-dependent inhibition on aberrant crypt foci and increase in the number of apoptotic cells in a period of 10 weeks, indicating a potential

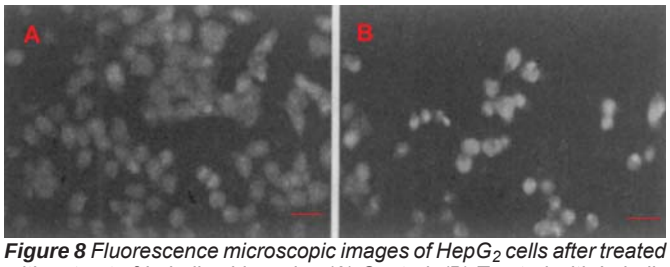


Figure 8 *Fluorescence microscopic images of $HepG_2$ cells after treated with extract of Lobelia chinensis. (A) Control; (B) Treated with Lobelia chinensis extract. (Taken from the work of Zhang et al)* [18]

effect on both the prevention and treatment of colon cancer. In the research of Ren *et al,* some piperidine alkaloids isolated from *L. chinensis* also exhibited moderate cytotoxic activities when tested against two human lung cancer cell lines, NCI-H292 and MSTO-211H.[9] More and more evidences show that *L. chinensis* has potential anti-cancer actitivity.

Choleretic effect

He *et al* found the intravenous administration of a raw *L.chinensis* made solution could increase bile secretion in the experimented dogs.[20] The follow-up clinical trials on patients with gallstones were conducted later. After the oral administration of *L. chinensis* decotion, the bile viscosity coefficients of the tested patients were significantly decreased. All of these results demonstrated the choleretic effect of this herbal medicine.

Other activities

In addition to the pharmacological activities introduced above, many other bioactivities of this herbal medicine have been detected and investigated also. For example, two piperidine alkaloids extracted from *L. chinensis* showed as inhibitors to α-glucosidase,[3] the intravenously administrated decoction and alkaloid preparation of *L. chinensis* excited the respiratory system in anesthetized dogs by stimulating the chemoreceptors in the carotid and aortic bodies,[21] the herbal decoction of *L. chinensis* protected mice from injected cobra venom when given orally 30 min prior at the minimum lethal dosage,[1] and *L. chinensis* exhibited obvious analgesic effect in mouse acetic acid-induced twisting test and hot plate test.[16]

SAFETY EVALUATIONS/CONTRAINDICATIONS

In fact, there is little report about the safety evaluation of *L. chinensis*. In the famous Chinese medicinal book "Ben Cao Gang Mu", *L. chinensis* was considered to be nontoxic. In another Chinese local medicinal book "Jiang Xi Min Jian Cao Yao Yan Fang", asthenia syndrome, which is called as "xu zheng" in Chinese, was considered as the contraindication of *L. chinensis* in TCM. In the test of *in vitro* and *in vivo* anti-virus activities with the methanolic extract from *L. chinensis*, no apparent toxic effects of this herbal medicine on liver or kidney functions was found by Chang *et al* in 2008.[6] Up to now, there is no specific scientific evidence about the contraindication or side effect of *L. chinensis* has been found.

CONCLUSIONS

There are many species of plants in *Lobelia* genus in nature. It is different from other species only used in folk medicine or local area, *L. chinensis* is listed in Chinese Pharmacopoeia and widely utilized as a conventional herbal medicine in China for a long time.[5] This traditional herbal medicine is principally used for the treatment of inflammation, snake-biting, jaundice with damp-heat pathogen and eczema tetter in TCM. For further study the chemical constituents of *L. chinensis*, large numbers of piperidine alkaloids and flavonoids as the major constituents in this plant have been successfully isolated and identified with the help of modern analytical technology.[2, 9, 10, 13, 14] At the same time, obvious biological activities, such as anti-virus and anti-cancer activities, of the extract and the isolated compounds from *L. chinensis* were also found in pharmacological studies and indicated some potential medical applications in modern medicine.[4,6-8, 16-21] However, *L. chinensis* is totally different from *L. inflate*, another species in *Lobelia* genus which is also called as Indian tobacco and commonly used for medical treatment of asthma, compared from botany property, place of original, part usually used, major chemical constituents and major applications (Table 1).[22, 23] As the major bioactive constituent of *L. inflate*, lobeline has not been detected in the tested samples of *L. chinensis* in our research.[11, 12] Finally, more studies about *L.chinensis* should be carried out to better utilize this valuable herbal resource in life science field in the future.

Table 1 Comparison of *Lobelia chinensis* and *Lobelia inflate*.

Differences	*Lobelia chinensis*	*Lobelia inflata*
Botany property	Small perennial plant, 15-35 cm tall	Annual or biennial plant, 15-100 cm tall
Place of Origin	East Asia including China, Japan and Korea	Eastern North America including America and Canada
Part Usually Used	Whole dried plant	Dried above ground part
Major Chemical Constituents	Piperidine alkaloids (no lobeline detected) and flavonoids	Piperidine alkaloid lobeline was considered as the major bioactive compound
Major Applications	Medical treatment of inflammation and snake-biting	Medical treatment of asthma, also used for antismoking due to the similar effect of lobline as nicotine

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Author's background

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References

1. Zhao ZZ, Xiao PG (2009). Encyclopedia of Medicinal Plants. World Publishing Corporation. Vol 2, p47-50.
2. Shibano M, Tsukamoto D, Masuda A, Tanaka Y, Kusano G (2001). Two new pyrrolidine alkaloids, radicamines A and B, as inhibitors of α-glucosidase from *Lobelia chinensis* Lour. ***Chemical Pharmaceutical Bulletin,*** 49(10): 1362-1365.
3. Chen JX, Huang SH, Wang L, Han WL, Wang Y, Zhang DM, Ye WC (2010). Two pairs of enantiomeric neolignans from *Lobelia chinensis*. ***Natural Product Communications,*** 5(10):1627-1630.
4. Kuo PC, Hwang TL, Lin YT, Kuo YC, Leu YL (2011). Chemical constituents from Lobelia chinensis and their anti-virus and anti-inflammatory bioactivities. ***Arch Pharmaceutical Research,*** 34(5): 715-722.
5. Committee NP (2010). Pharmacopoeia of the People's Republic of China. p109.
6. Kuo YC, Lee YC, Leu YL, Tsai WJ, Chang SC (2008). Efficacy of orally administered Lobelia chinensis extracts on herpes simplex virus type 1 infection in BALB/c mice. ***Antiviral Research,*** 80(2): 206–212.
7. Miller DK, Lever JR, Rodvelt KR, Baskett JA, Will MJ, Kracke GR (2007). Lobeline, a potential pharmacotherapy for drug addiction, binds to μ-opioid receptors and diminishes the effects of opioid receptor agonists. ***Drug and Alcohol Dependence,*** 89(2-3): 282–291.
8. Chen MW, Chen WR, Zhang JM, Long XY, Wang YT (2014). Lobelia chinensis: chemical constituents and anticancer activity perspective. ***Chinese Journal of Natural Medicines,*** 12(2):0103-0107.
9. Yan S, Shen T, Zhao L, Li C, Zhang Y, Lou H, Ren D (2014). Chemical constituents of Lobelia chinensis. ***Fitoterapia***, 93: 168-174.
10. Kuang JX, Liu LZ, Ye F (2010). Study on optimum extraction process of the total alkaloids in the Lobelia chinensis with orthogonal design. ***Chinese Jouranl of Ethnomedicines & Ethnopharmacology,*** 19(1): 43-44.
11. Felpin FX, Lebreton J (2006). History, chemistry and biology of alkaloids from *Lobelia inflate*. ***Tetrahedron,*** 60(45): 10127-10153.
12. Kursinszki L, Ludányi K, Szöke E (2008).LC-DAD and LC-MS-MS analysis of piperidine alkaloids of Lobelia inflate L. (*in vitro* and *in vivo*). ***Chromatographia,*** 68(1): S27-33.
13. Huang XX, Wei HL, Pan YF (2012). Study on ultrasonic wave-assisted semi-bionic extraction and antioxidant activity of flavonoid glycoside from herb *Lobelia chinensis*. ***Sciences & Technologies of Food Industry,*** 34(3): 59-61.
14. Jiang Y, Shi R, Liu B, Wang Q, Dai Y(2009). Studies on chemical components of *Lobelia chinensis*. ***China Journal of Chinese Materia Medica,*** 34(3): 294-297.
15. Qiao CF, He ZD, Han QB, Song JZ, Xu HX (2006). Determination of two polyacetylenes in herba *Lobeliae chinensis* by HPLC analysis. ***China Journal of Chinese Materia Medica,*** 31(9): 744-746.
16. Huang LD, Guo LQ, Pan TQ, Pan XY, Yan ZD, Liu SL, Zhao SM, He XJ (2012). Experimental study on anti-inflammatory and analgesic effects of different extracts from Chinese Lobelia. ***Herald of Medicine,*** 31(8): 982-985.
17. Wang JJ, Fan XZ, Liu SM, Ren DM, Chen R, Li L, Hu WC(2006). Inhibitory effects of Lobelia chinensis Lour alkaloid on the proliferation of human umbilical artery vascular smooth muscle induced by endothelin-1. ***Chinese Journal of Pathophysiology***. 22(1):26-30.
18. Gao D, Liu RY, Zhang ZL 2006. Experimental study of hepatoma cells apoptosis induced by *Lobelia chinensis* through calcium pathway. ***Journal of Fujian College of TCM,*** 16(6): 32-33.
19. Han SR, Lv XY, Wang YM, Gong H, Zhang C, Tong AN, Yan N (2013). A study on the effect of aqueous extract of *Lobelia chinensis* on colon precancerous lesions in rats. ***African Journal of Traditional & Complement ary Alternative Medicines,*** 10(6): 422-425.
20. Liu S, Liu XY, Tang HH, He ZJ (1995). An experimental study and clinical trial on banbianlian's effect of promotion of bile secretion. ***China Journal of Modern Medicine***., 5(3): 1-2.
21. Yan BF (2003). Pharmcology. Beijing: People's Medical Publishing House.p134.
22. Stansbury J, Saunders, PR, Zampieron ER (2013). The use of lobelia in the treatment of asthma and respiratory illness. ***Journal of Restorative Medicine,*** 2(1): 94-100.
23. Hughes PW, Jaworski AF, Davis CS, Aitken SM, Simons AM (2014). Development of polymorphic microsatellite markers for Indian tobacco, *Lobelia inflate* (Campanulaceae). ***Applications in Plant Sciences,*** 2(4): 1300096.

REFERENCES: 1. Vasar M et al. Fluticasone furoate nasal spray: Effective monotherapy for symptoms of perennial allergic rhinitis in adults/adolescents. Allergy Asthma Proc 2008; 29(3): 313–321 **2.** Maspero JF et al. The efficacy of fluticasone furoate on 7 individual nasal and ocular symptoms of seasonal allergic rhinitis. Abstract 524, World Allergy Congress, Buenos Aires, December 2009 **3.** Sardana N et al. Congestion and Sleep Impairment in Allergic Rhinitis. Asian Pac J Allergy Immunol 2011; 29:297–306 **4.** HKAPI, HK Sales data in Class R1A, R1B, R6A, 2011 to Mar 2013 **5.** Meltzer EO et al. Preferences of Adult Patients with Allergic Rhinitis for the Sensory Attributes of Fluticasone Furoate Versus Fluticasone Propionate Nasal Sprays: A Randomized, Multicenter, Double-Blind, Single-Dose, Crossover Study. Clin Ther 2008; 30: 271–279 **6.** Avamys Hong Kong Prescribing Information, 2012

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HKRX/FF/0010/13 (07/2015)

HONG KONG PHARMACY CONFERENCE
香港藥劑學術年會
www.pharmacyconference.org

P.O. Box 90155, Tsim Sha Tsui, Kowloon, Hong Kong

CUHK DH HA HKU PSHK PPA SHPHK

Dear Pharmacists, friends and colleagues,

Being one of the biggest events in the pharmacy profession of Hong Kong, the Hong Kong Pharmacy conference will be held on the 28th to 29th March 2015 at the Hong Kong Convention and Exhibition Centre. The Hong Kong Pharmacy Conference is an event gathering pharmacists from all sectors of practice. This year is of no exception. Same with previous years, the conference is jointly organised by the School of Pharmacy of the Chinese University of Hong Kong, the Department of Health, the Hospital Authority, the Department of Pharmacology and Pharmacy in the University of Hong Kong, the Practicing Pharmacists Association of Hong Kong, the Pharmaceutical Society of Hong Kong and the Society of Hospital Pharmacists of Hong Kong. On behalf of the organising committee, I would like to extend our warm welcome to all of you to the Hong Kong Pharmacy Conference 2015.

This year, we have put an extra effort to create a broader platform for pharmacists to share their knowledge and expertise in different practice sectors. Although pharmacists practising in different sectors can contribute to the society in a very different way, we all have one common goal – to excel our professional practice and to provide better quality of care. We, therefore, claim our theme this year to be "Harmony and Diversity – Through Partnership and Leadership".

The conference will start with the inspiration of leaders. We are honoured to have invited the Under Secretary of the Food and Health Bureau, Professor Sophia Chan, JP to give us a speech about leadership and inter-professional collaboration. We have also invited the leading pharmacists from UK and Singapore to talk about their experiences in establishing new services in paediatric and oncology. With the directional insight and practice inspiration of theme speeches, the concurrent sessions on day two would continue to discuss how the diversity of practices to be led.

Unlike previous years, we have made our concurrent sessions in line with our theme. We have "Leadership", "Partnership" and "Diversity" as three concurrent sessions on Day two. We would have pioneers to stimulate our creativities in practices by sharing with us some outstanding practices in the leadership session. Pharmacists from community, hospital and industrial practice would also show us how they cooperate with different health care professionals to deliver the high quality of patient care. In the diversity session, we would have pharmacists to discuss how their knowledge to be applied to a diversity of patient populations. We have updates on the treatment of Hepatitis C as well as the use of the New Oral Anti-Coagulants (NOACs). We also have practice discussion on the use of Traditional Chinese Medicine (TCM) and health food product. We hope pharmacists with different interests in their practices can immerse themselves in the sharing and the discussion.

To wrap up the conference with a large variety of content, we would feature a plenary session discussing the current issue of the pharmacy practice with the heads of seven organising institutes. It is a great opportunity to keep abreast of the current changes in our profession.

I am looking forward to seeing you in the Hong Kong Pharmacy Conference 2015.

Yours faithfully,

Ritchie Kwok

Hong Kong Pharmacy Conference 2015

Please visit our website for the most updated information. http://www.pharmacyconference.org/

The 25th Federation of Asian Pharmaceutical Association (FAPA) Congress

WONG, Anabelle Ngachung[a] ; YAU, Edward[b]

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The 25th Federation of Asian Pharmaceutical Association (FAPA) Congress held in Kota Kinabalu, Sabah, Malaysia, focused on expanding the pharmacists' role in wellness and sustainable health. 8 plenary lectures and 18 concurrent symposia held by speakers from not only all over Asia, but also from as far as the United Kingdom and Australia; 114 concurrent presentations and 184 study posters on various topics related to hospital and clinical pharmacy, scientific findings, pharmaceutical education and student affairs, industrial pharmacy, community pharmacy, pharmaceutical ethics and regulatory affairs, phytopharmacy and pharmacopoeia, and emergency medicines; and several warm and welcoming social events, such as the friendship night and the gala dinner; were packed into 9th-12th October, 2014. It was undoubtedly an eye-opening experience for young pharmacists and pharmacy students; a nourishing programme for practising colleagues from all sectors; and a golden occasion for scholars and lecturers to share their expertise in different fields with guests yearning for knowledge. (Photos 1 to 4)

Photo 1: *25th FAPA Congress at Kotakinabalu from 9th to 11th October 2014*

Photo 2: *Over 180 poster presentations.*

Photo 3: *Mrs. Mary Catherine Cheng, President of PSHK representing Hong Kong in the opening ceremony.*

Photo 4: *Young pharmacists Group and Hong Kong delegates on friendship night at Hakka Hall.*

Representing Hong Kong, Mrs. Mary Catherine Cheng, the president of the Pharmaceutical Society of Hong Kong (PSHK), accompanied by Mr. Ken Cheng; and two members of the PSHK, Edward F. W. Yau and Anabelle N. Wong, participated in the 25th FAPA Congress with Prof Cheung Hon Yeung, who was given the Ishidate Award; and Prof.Vivian W. Y. Lee, who was invited to hold a symposium. (Photo 5)

Photo 5: *(from left to right) Ms. Anabelle Wong, Mr. Edward Yau, Mrs. Mary Catherine Cheng, Prof. Cheung Hon Yeung, Prof. Vivian Lee*

The 25th FAFA Congress kicked off on 9th October with the poster exhibition opening, followed by the first plenary lecture by Dr. Salmah binti Bahri from Malaysia on Pharmacists Today – Winds of Change through the Decades, in which the evolvement of pharmacists' role and responsibility as well as the direction of the profession's future development was discussed; and the second lecture by Mr. Ashock Soni from the United Kingdom on Global Innovative Strategies to Expand Pharmacists' Roles in Wellness and Sustainable Health, in which the shift of patient care from tertiary care to primary care was advocated; the importance of providing innovative services was reinforced; and the idea of the Royal Pharmaceutical Society in seeking to make all pharmacists prescribers in the near future was shared. The first day of Congress ended with the opening ceremony. The cocktail reception after the opening ceremony was when the representative from the Young Pharmacist Group (YPG) in Singapore invited the representatives from Hong Kong to sit in the Asian Young Pharmacist Group (AYPG) Meeting on 10th October.

Following 3 plenary lectures and a special symposium, Prof. Cheung Hon Yeung, one of the Ishidate Awardees, gave a lecture titled, "Trends of Pharmacy Education and Trainings in Asia: Proper Transformation or Not?" When asked about his comments on the Congress and being awarded, Prof. Cheung said that he was happy to participate in the FAPA Congress and thankful for the nomination by Ms. Mary Catherine Cheng for the Ishidate award in Education.

(Photo 6) Concurrently, Dr. Vivian Lee held a symposium for Pharmacy Education for Sustainable Tomorrow – Asia Experience.

Photo 6: *Prof. Cheung Hon Yeung receiving the Ishidate Award on Education*

Meanwhile, Mr. Edward Yau and Ms. Anabelle Wong were attending the AYPG meeting. In spite of being an observing organisation, it was Hong Kong's first involvement in the AYPG. The AYPG was set up by a group of young pharmacists in 2011 and the first council meeting was held in September 2012 during the FAPA congress in Bali. The group is not officially part of the FAPA framework but is in close relations with the Bureau, where their council meetings are held during the FAPA congress. Young Pharmacists Groups from the Asia region unite to form this organization for professional networking, experience sharing, and most importantly, some fun and laughter together.

During the 2nd AYPG Council & Business Meeting held on the second day of the FAPA congress this year, Mr. Alston Tsai from Taiwan has assumed the position of President of the AYPG, with Mr. Jack Shen Lim from Malaysia as Immediate Past President and Mr. Bryan Posadas from Philippines as the President-Elect. There are three vice-presidents elected in the meeting and they are Ms. Audrey Clarissa from Indonesia, Ms. Yoko Nanaumi from Japan, and Mr. Anson Lim from Singapore. This makes up the total of seven member organizations which are part of the current AYPG. Both Cambodia and Hong Kong attended the meeting as observers. (Photo 7)

Photo 7: *Young Pharmacists Group on friendship night at Hakka Hall*

One of the main goals of the AYPG is to help countries in the region to set up their own YPG. As they have vast experiences to share in the development and challenges of setting up such groups. YPG can take various formats and framework to fit the needs and demands of each country. Taiwan for example has a large number of active young pharmacists who are part of the Taiwan YPG, and they have an independent organization which is separate from their Pharmaceutical Society. Singapore, which is the newest member organization of the AYPG, has an YPG which is attached to the Pharmaceutical Society of Singapore.

Delegates from the Japan YPG gave an interesting presentation on the history of the pharmacy profession, and the effort in which Japanese pharmacists have given in their long journey to exclusive dispensing rights. Their challenges had been many, and previous generations of pharmacists have fought hard to raise awareness to the public on their hopes and aspirations to become the guardian of medicines. Japanese pharmacists were formally recognized as a health professional finally by a change in regulation, but their journey continues. At the moment there are several members of their parliament who are pharmacists and are able to voice out on issues related to pharmacists and the healthcare profession.

On the lighter side, the AYPG has announced the establishment of a travel bureau, where a travel network would be established for pharmacists within the group. Pharmacists within the group will now be able to travel to another country within the AYPG and have the warm reception of pharmacists of the host country. The host pharmacists would have local knowledge and be able to give good travel advice and possibly help make some arrangements if necessary. It is expected that other member countries would return the favour and become the host in the future. This is a great way to network while travelling and is one of the best perks of becoming part of the AYPG family.

The next formal event of the group will be a leadership forum hosted in Taipei in November next year. Details of the event shall be release closer to the time and pharmacists from within the group and observer organizations would be able to join.

Having absorbed the latest knowledge from the plenary lectures and concurrent presentations like sponges; and inspired by the idea in shaping the niche and thrusting the development of pharmacy in Asia, all guests were treated to some fine Malaysian cuisine in the Hakka Hall sponsored by the state government in Sabah at the friendship night. It was a delightful event for healthcare professionals from different regions to network and to understand the cultures and customs of different regions better. It also provided a showcase for the talented pharmacists, who might be singers or dancers, all of whom knew well how to explode rounds and rounds of applause.

The Congress goers were enlightened with Australian experience in applying the cutting edge robotic technology in offering innovative pharmacy service the morning after. The concept of Expanding Pharmacists' Role in the Humanitarian and Disaster Relief (HADR) Mission was shared through Malaysian Military Pharmacy Perspectives by Col Dr. A Halim Hj. Robotic technology in pharmacy has been introduced in Hong Kong for years, yet its use has not been maximised and a considerable portion of manpower and time is still spent on dispensing. Larger steps forward in pharmacy automation in Hong Kong are therefore hoped for. Although the idea of pharmacists' contribution in humanitarian aids units is not a new thought to the world, little possibility is explored in Hong Kong's setting. Most graduates from local Pharmacy Schools desire to work for hospitals, community pharmacies, or pharmaceutical companies; fewer would enter local regulatory authority or dedicate to research. Seldom has non-governmental organisation (NGO) been mentioned in graduates' career plans, if not never.

Are Hong Kong's younger generations of pharmacists up for challenges? How eager are they in expanding the role of pharmacists in sustainable health in Hong Kong? "No decision about me without me," said the patients in Liberating the NHS. What a wakening statement, a statement that everyone has no reason to hesitate from making.

MIRAPEX® ER
(Boehringer Ingelheim)

Prepared by Fiona Yuen and edited by Ivy Chan

Active Ingredient:
Pramipexole

Presentation:
Mirapex ER 0.375mg – Each extended-release tablet contains 0.26 mg of pramipexole base

Mirapex ER 0.75mg – Each extended-release tablet contains 0.52 mg of pramipexole base

Mirapex ER 1.5mg – Each extended-release tablet contains 1.05 mg of pramipexole base

Pharmacological Properties:
Pramipexole, the active ingredient of MIRAPEX, is a dopamine agonist and binds with high selectivity and specificity to the dopamine D2 subfamily receptors and has a preferential affinity to D3 receptors; it has full intrinsic activity.

MIRAPEX alleviates parkinsonian motor deficits by stimulation of dopamine receptors in the striatum. Animal studies have shown that pramipexole inhibits dopamine synthesis, release, and turnover.

In human volunteers a dose-dependent decrease in prolactin was observed. In a clinical trial with healthy volunteers, where MIRAPEX extended-release tablets were titrated faster than recommended (every 3 days) up to 4.5 mg per day, an increase in blood pressure and heart rate was observed. Such effect was not observed in patient studies.

Parkinson's disease:

Efficacy of MIRAPEX in the controlled clinical trials was maintained for the duration of the trials, approximately six months. In open continuation trials lasting for more than three years there were no signs of decreasing efficacy.

The efficacy and tolerability of an overnight switch from MIRAPEX tablets to MIRAPEX extended-release tablets at the same daily dose was evaluated in a double-blind clinical study in patients with early Parkinson's disease.

Efficacy was maintained in 87 of 103 patients switched to MIRAPEX extended-release tablets. Out of these 87 patients, 82.8% did not change their dose, 13.8% increased and 3.4% decreased their dose. In half of the 16 patients who did not meet the criterion for maintained efficacy on UPDRS Part II+III score, the change from baseline was considered not clinically relevant.

One patient switched to MIRAPEX extended-release tablets experienced a drug-related adverse event leading to withdrawal.

Indications:
For the treatment of signs and symptoms of idiopathic Parkinson's disease. It may be used as monotherapy or in combination with levodopa.

Dosage & Administration:
All dose information refers to pramipexole salt form

Parkinson's disease

The extended-release tablets should be taken once daily at about the same time each day. The extended- release tablets should be swallowed whole with water, and must not be chewed, divided or crushed. The extended- release tablets may be taken with or without food.

When the intake of a dose is missed, MIRAPEX extended-release tablets should be taken up to 12 hours after the regularly scheduled time. After 12 hours, the missed dose should be left out and the next dose should be taken on the following day at the next regularly scheduled time.

Initial treatment:

As shown below dosages should be increased gradually from a starting dose of 0.375 mg per day and then increased every 5 - 7 days. Providing patients do not experience intolerable side effects, the dosage should be titrated to achieve a maximal therapeutic effect.

Ascending-Dose Schedule of MIRAPEX extended-release tablets	
Week	***Total daily dose (mg)***
1	0.375
2	0.75
3	1.50

If a further dose increase is necessary the daily dose should be increased by 0.75 mg at weekly intervals up to a maximum dose of 4.5 mg per day.

Patients already taking MIRAPEX tablets may be switched to MIRAPEX extended-release tablets overnight, at the same daily dose.

Maintenance treatment:

The individual dose should be in the range of 0.375 mg to a maximum of 4.5 mg per day. During dose escalation in pivotal studies both in early and advanced disease efficacy was observed starting at a daily dose of 1.5 mg. This does not preclude that in individual patients doses higher than 1.5 mg per day can result in additional therapeutic benefit.

This applies particularly to patients with advanced disease where a reduction of the levodopa therapy is intended.

Treatment discontinuation:

MIRAPEX extended-release tablets should be tapered off at a rate of 0.75 mg per day until the daily dose has been reduced to 0.75 mg. Thereafter the dose should be reduced by 0.375 mg per day.

Dosing in patients with concomitant levodopa therapy:

In patients with concomitant levodopa therapy it is recommended that the dosage of levodopa is reduced during both dose escalation and maintenance treatment with MIRAPEX. This may be necessary in order to avoid excessive dopaminergic stimulation.

Dosing in patients with renal impairment:

The elimination of pramipexole is dependent on renal function. The following dosage schedule is suggested for initiation of therapy:

Patients with a creatinine clearance above 50 ml/min require no reduction in daily dose or dosing frequency.

In patients with a creatinine clearance between 30 and 50 ml/min, treatment should be started with 0.375 mg MIRAPEX extended-release tablets every other day. Caution should be exercised and careful assessment of therapeutic response and tolerability should be made before increasing to daily dosing after one week. If a further dose increase is necessary, daily doses should be increased by 0.375 mg at weekly intervals up to a maximum dose of 2.25 mg per day.

The treatment of patients with a creatinine clearance below 30ml/min with MIRAPEX extended-release tablets is not recommended as no data are available for this patient population. The use of MIRAPEX tablets should be considered.

If renal function declines during maintenance therapy the recommendations given above should be followed.

Dosing in patients with hepatic impairment:

Dose adjustment in patients with hepatic failure is probably not necessary, as approx. 90% of absorbed active substance is excreted through the kidneys. However, the potential influence of hepatic insufficiency on MIRAPEX pharmacokinetics has not been investigated.

Contraindications:
Hypersensitivity to pramipexole or any other component of the product.

Precautions:
When prescribing MIRAPEX in a patient with renal impairment a reduced dose is suggested in line with section Dosage & administration.

Hallucinations and confusion are known side effects of treatment with dopamine agonists and levodopa in Parkinson's disease patients. Hallucinations were more frequent when MIRAPEX was given in combination with levodopa in Parkinson's disease patients with advanced disease than in monotherapy in Parkinson's disease patients with early disease. Within the RLS clinical development program for registration, one case of hallucinations has been reported. Patients should be informed that (mostly visual) hallucinations can occur.

Patients should be aware of the fact that hallucinations can occur and may adversely affect their ability to drive.

Patients and caregivers should be aware of the fact that abnormal behaviour (reflecting symptoms of impulse control disorders and compulsive behaviours) such as binge eating, compulsive shopping, hypersexuality and pathological gambling have been reported in patients treated with dopaminergic drugs. Dose reduction/tapered discontinuation should be considered.

Patients with psychotic disorders should only be treated with dopamine agonists if the potential benefits outweigh the risks. Co-administration of antipsychotic medicinal products with pramipexole should be avoided, eg. if antagonistic effects can be expected.

Pathologic changes (degeneration and loss of photoreceptor cells) were observed in the retina of albino rats in the 2-years carcinogenicity study. Evaluation of the retinas of albino mice, pigmented rats, monkeys, and minipigs did not reveal similar changes. The potential significance of this effect in humans has not been established, but cannot be disregarded because disruption of a mechanism that is universally present in vertebrates (i.e. disk shedding) may be involved.

Ophthalmologic monitoring is recommended at regular intervals or if vision abnormalities occur.

In case of severe cardiovascular disease, care should be taken. It is recommended to monitor blood pressure, especially at the beginning of treatment, due to the general risk of postural hypotension associated with dopaminergic therapy.

Patients should be alerted to the potential sedating effects associated with MIRAPEX, including somnolence and the possibility of falling asleep while engaged in activities of daily living. Since somnolence is a frequent adverse event with potentially serious consequences, patients should neither drive a car nor operate other complex machinery until they have gained sufficient experience with MIRAPEX to gauge whether or not it affects their mental and/or motor performance adversely. Patients should be advised that if increased somnolence or episodes of falling asleep during activities of daily living (e.g. conversations, eating, etc.) are experienced at any time during treatment, they should not drive or participate in potentially dangerous activities and should contact their physician.

Epidemiological studies have shown that patients with Parkinson's disease have a higher risk (2- to approximately 6-fold higher) of developing melanoma than the general population. Whether the increased risk observed was due to Parkinson's disease or other factors, such as drugs used to treat Parkinson's disease, is unclear.

For the reasons stated above, patients and providers are advised to monitor for melanoma when using pramipexole or other dopaminergic drugs.

Parkinson's disease

Symptoms suggestive of a neuroleptic malignant syndrome have been reported with abrupt withdrawal of dopaminergic therapy.

Drug Interactions:
Pramipexole is bound to plasma proteins to a very low (< 20%) extent and little biotransformation is seen in man. Therefore, interactions with other medication affecting plasma protein binding or elimination by biotransformation are unlikely.

Medication that inhibit the active renal tubular secretion of basic (cationic) drugs, such as cimetidine, or are themselves eliminated by active renal tubular secretion, may interact with MIRAPEX resulting in reduced clearance of either or both medication. In case of concomitant treatment with these kinds of drugs (incl. amantadine) attention should be paid to signs of dopamine over stimulation, such as dyskinesias, agitation or hallucinations. In such cases a dose reduction is necessary.

Selegiline and levodopa do not influence the pharmacokinetics of pramipexole. The overall extent of absorption or elimination of levodopa is not changed by pramipexole. The interaction with anticholinergics and amantadine has not been examined.

As anticholinergics are mainly eliminated by hepatic metabolism, pharmacokinetic drug-drug interactions with pramipexole are rather unlikely. With amantadine, an interaction is possible via the same system of excretion in the kidney.

While increasing the dose of MIRAPEX in Parkinson's disease patients it is recommended that the dosage of levodopa is reduced and the dosage of other antiparkinsonian medication kept constant.

Because of possible additive effects, caution should be advised when patients are taking other sedating medication or alcohol in combination with MIRAPEX and when taking concomitant medication that increase plasma levels of pramipexole (e.g. cimetidine).

Side Effects:
The following side effects are listed under the use of MIRAPEX: abnormal behaviour (reflecting symptoms of impulse control disorders and compulsions) such as binge eating, compulsive shopping, hypersexuality and pathological gambling; abnormal dreams, amnesia, cardiac failure, confusion, constipation, delusion, dizziness, dyskinesia, dyspnoea, fatigue, hallucinations, headache, hiccups, hyperkinesia, hyperphagia, hypotension, inappropriate antidiuretic hormone secretion, insomnia, libido disorders, nausea, paranoia, peripheral oedema; pneumonia; pruritus, rash and other hypersensitivity; restlessness, somnolence, sudden onset of sleep, syncope, visual impairment including diplopia, vision blurred and visual acuity reduced, vomiting, weight decrease including decreased appetite, weight increase.

The incidence of hypotension under MIRAPEX, compared to placebo treatment, was not increased. However, in individual patients, hypotension may occur at the beginning of treatment, especially if MIRAPEX is titrated too rapidly.

MIRAPEX may be associated with disorders of libido (increase or decrease).

Patients treated with pramipexole tablets have reported falling asleep during activities of daily living, including the operation of motor vehicles, which sometimes resulted in accidents. Some of them did not report a warning sign such as somnolence, which is a common occurrence in patients receiving pramipexole tablets at doses above 1.5 mg/day, and which, according to the current knowledge of sleep physiology, always proceeds falling asleep. There was no clear relation to the duration of treatment. Some patients were taking other medication with potentially sedative properties. In most cases where information was available, there were no further episodes following reduction of dosage or termination of therapy.

Patients treated with dopamine agonists for Parkinson's disease, including MIRAPEX, especially at high doses, have been reported as exhibiting signs of pathological gambling, increased libido and hypersexuality, generally reversible upon reduction of the dose or treatment discontinuation.

In clinical studies and post-marketing experience cardiac failure has been reported in patients with pramipexole. In a pharmacoepidemiological study pramipexole use was associated with an increased risk of cardiac failure compared with non-use of pramipexole. A causal relationship between pramipexole and cardiac failure has not been demonstrated.

Forensic Classification:
P1S1S3

31% of men may have premature ejaculation, which can severely impact quality of life for them and their partners.[1] Priligy® (dapoxetine) was developed specifically for the treatment of premature ejaculation.[2,3] and is clinically proven to be effective and well-tolerated.[4,5]

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References

1. McMahon CG, Lee G, Park JK, Adaikan PG. Premature ejaculation and the erectile dysfunction prevalence and attitudes premature ejaculation and Asia-Pacific region. J Sex Med. 2012;9:424-465.
2. Wespes E, Eardley AE, Giuliano F, Hatzichristou D, Hatzimouratidis K, Montorsi F, et al. European Association of Urology: Guidelines Male Sexual Dysfunction: Erectile dysfunction and premature ejaculation. [Internet] 2012 Feb [cited 2013 Mar 4]. Available from: http://www.uroweb.org/fileadmin/guidelines/2012_Guidelines_large_text_print_total_file.pdf.
3. Andersson KE, Mulhall JP, Wyllie MG. Pharmacokinetic and pharmacodynamic features of dapoxetine, a novel drug for 'on-demand' treatment of premature ejaculation. BJU Int. 2006;97(2):311-5.
4. McMahon CG, Althof SE, Kaufman JM, Buvat J, Levine SB, Aquilina JW, et al. Efficacy and safety of dapoxetine for the treatment of premature ejaculation: integrated analysis of results from five phase 3 trials. J Sex Med. 2011;8:524-539.
5. McCarty EJ, Dinsmore W. Dapoxetine: an evidence-based review of its effectiveness in treatment of premature ejaculation. Core Evid. 2012;7:1-14.

In order to ensure that Priligy is used appropriately and to avoid the risk of syncope, an appropriate use guide for physicians and an information brochure for patients are available from A. Menarini. It is strongly recommended that physicians obtain and read these risk management educational materials before prescribing Priligy. The materials can be ordered from A. Menarini on 3605 5888 or may be obtained from your local A. Menarini representative.

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