

HONG KONG PHARMACEUTICAL *JOURNAL*

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Student's Experience on Attending the 2018 Forbidden City International Pharmacist Forum

SHPHK – New Term, New Vision

The Society of Hospital Pharmacists of Hong Kong (SHPHK) Office Bearers 2018/2019

The Drug Education Resources Centre (DERC) Office Bearers 2018/19

PSHK Upcoming Continuing Education Programmes for Pharmacists

KEYTRUDA® (MSD)

ZEPATIER® (MSD)



*The Pharmaceutical Society of Hong Kong
The Practising Pharmacists Association of Hong Kong
The Society of Hospital Pharmacists of Hong Kong*

A Silver Jubilation of Publication: It is Time to be Celebrated for HKPJ



I am proud to present Volume 25, issue 2 of Hong Kong Pharmaceutical Journal.

The Pharmaceutical Society of Hong Kong (PSHK) was found in 1949.⁽¹⁾ I am sure members of the society are going to celebrate its 70th Anniversary of founding in the coming year. But don't forget this year also marks a profound breakthrough in history of the society; i.e. launching the

publication of Hong Kong Pharmaceutical Journal (HKPJ) by the society some 25 years ago. Publication of a professional journal signified the objectives of our society to promote the professional characteristic, scientific bases and educational commitment of our pharmacy practices.

Throughout this quarter of a century, I have witnessed many aspects of progress of this journal; in particular, on professional and printing quality. I have to say some members of our society have been faithfully and persistently devoted their effort to make this journal regularly published even though they have increasing responsibilities to their family and works. Although occasionally there were one or two issue either discontinued or combined due to various reasons, it has not been totally halted. Because of the authors and contributors' effort, it makes the publication of this journal last until these days.

It has always been my vision that a professional society, like PSHK, should be built on solid foundation of characters that her activities should be documentary-based, scientific approach and promoting life-time learning. Because of this wish, it gives me the drive and strength that explains why I could associate with this journal for so long. This issue signifies my involvement in editing two sections, namely the Pharmaceutical Techniques & Technology section and the section of Herbal Medicines & Nutraceuticals at the beginning for nearly a quarter of century and subsequently servicing as the Editor-in-Chief for ten years to this journal on voluntary bases. Throughout this period of involvement, I have witnessed people coming in and leaving to the editorial board. Some quit their involvement as short as one issue after briefed. I could understand why people quit because using a pen to write is not an easy task for most people; it requires persistent devotion, commitment, knowledge, willingness to share on top of good trainings in presentation skill. I am not a particularly gifted person either except my fond of pharmacy that drives me to spend so many hours on practicing various aspects of running a journal. Certainly, I also have to contribute many original research results to this journal in order to keep it running without considering any career rewards. I dare to say I have already given my best to my profession.

But after so many years of holding the editorial, it is time for me to have a break and let other to bring in some other new ideas so that this journal could akin to a higher land.

Content-wise, I am pleased to introduce three pieces of original research reports in this issue, i.e. firstly a paper present by Dr. Tsai *et al* on the design and production of a chimeric dimer molecule based on genetic engineering to cure colon cancer (page 46); Secondly, the analytical evaluation done by Huang *et al* to distinguish some confusing species of botanical materials for use as herbal medicines (page 43) and thirdly, clarification of an unsettled issue in the Chinese Medicine Ordinance (Cap. 549) of Hong Kong on the distribution of a bioactive component in different compartment of the *Crotonis Fructus* and its proper description (page 54). For the first one, it is a very good pieces of scientific report worthwhile every pharmacist to learn because it is about the latest tactic of drug discovery based on our understanding of molecular biology. This new dimer molecule, which has been patented and published,^(2,3) shuts down differentiation of the colon cancer via perturbation of the immuno- and angiogenesis responses of the tumor. Hence, it is a novel approach and may have significant impact on curing cancer. If you are interested on new drug discovery, you should go through it to get a glimpse how this new targeted drug is designed.

Besides these three original research reports, there is a review article on three recently registered cardiovascular drugs in Hong Kong (page 37). The authors provide a literature review on the anti-coagulative aspect of Bivalirubin and Edoxaban as well as an antidote for dabigatran, namely, Idarucizumab by comparing their indications, pharmacology, side effects and efficacy to some other drugs currently in use. I wish all readers can learn something new from them.

I hope you will take your time to read and make your efforts to contribute your original works or any review articles for publication in this journal.

Cheung Hon-Young

Editor-in-Chief

July 21, 2018

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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
- OTC & Health
- Medication Safety
- Society Activities
- Drugs & Therapeutics
- Pharmaceutical Techniques & Technology
- Herbal Medicines & Nutraceuticals
- 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.

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Prepared by Tommy Lee and Bryan Kan

Antidepressant Associated With Weight Gain

Date: April 13, 2018

Obesity is an increasing prevalent globally and puts enormous strain onto the healthcare system. There are studies on the association of obesity with depression, as well as with antidepressants. However, many of them are either limited in the trial setting, duration or sample size.

Therefore, a population based cohort study was carried out to evaluate the long term association between antidepressant prescription and body weight. It utilized the UK Clinical Practice Research Datalink (CPRD). Data of 2,006,296 adults aged 20 or above (registered from 2004 to 2014) was sampled (136,762 men and 157,957 women). The main outcomes were antidepressants prescription, incidence of weight gain ($\geq 5\%$), and transition to overweight or obesity. The rate ratios were estimated from a Poisson model and adjusted for covariates.

17,803 men (13.0%) and 35,307 women (22.4%) were found to have antidepressant prescribed in the year of study entry. The incidence of weight gain with antidepressant was significant (adjusted rate ratio (aRR): 1.21, 95% CI: 1.19-1.22) during follow-up. The risk continued to rise during the follow-up (for ≥ 6 years). There were significant risks of transition to overweight or obesity from normal weight (aRR: 1.29, 95% CI: 1.25-1.34), and transition to obesity from overweight (aRR: 1.29, 95% CI: 1.25-1.33).

The utilization of antidepressant is associated with increase in incidence of weight gain. However, the relationship may not be causal due to possible residual confounding effect.

Source: www.bmj.com

FDA Warning on Severe Immune System Reaction of Lamotrigine

Date: April 25, 2018

Lamotrigine is used to treat seizures in patients who are two-year-old or above. It is also indicated for maintenance treatment in patients with bipolar disorder. Recently, FDA warned that lamotrigine can cause a rare but serious reaction that can extensively activate the body's immune system. This can cause severe inflammation throughout the body and may result in death especially when it is not treated quickly.

The immune reaction, which is called hemophagocytic lymphohistiocytosis (HLH), typically presents as a persistent

fever, usually greater than 101oF. HLH can cause severe issues with the blood cells and organs such as liver, kidneys and lungs.

Prompt recognition and treatment are of paramount importance for improving the HLH outcomes and decrease mortality. Evaluate patients who develop fever or rash promptly and discontinue lamotrigine if HLH or another serious immune-related adverse reaction is suspected.

Source: www.fda.gov

Tafinlar®+Mekinist® Approved for BRAF-positive Anaplastic Thyroid Cancer

Date: May 04, 2018

The U.S. Food and Drug Administration (FDA) approved the co-administration of Tafinlar® (dabrafenib) and Mekinist® (trametinib) for the treatment of anaplastic thyroid cancer (ATC) that cannot be removed surgically or is metastatic, for patients with BRAF V600E mutation. This approval was granted to Novartis Pharmaceutical corporation after result from an open-label clinical trial.

ATC is an aggressive form of thyroid cancer, which account for about 1-2% of all thyroid cancers in the US. Tafinlar®/Mekinist® combination therapy is the first FDA-approved therapy for ATC. Aside from metastatic melanoma and non-small cell lung cancer, ATC is the third cancer with BRAF V600E mutation that the therapy is approved to treat by the FDA.

However, the side effects should be noted of. Both drugs may have risk of new cancer development (cutaneous squamous cell carcinoma). Meanwhile, co-administration Tafinlar®/Mekinist® can harm developing foetus, which requires effective contraceptive methods other than hormone therapy. The therapy may cause bleeding problem, gastrointestinal inflammation or tears, heart problem, severe skin reaction, hyperglycaemia, eye and breathing problem.

According to the Drug Office database, Mekinist® is available in Hong Kong as 0.5 or 2 mg tablets while Tafinlar® is available as 50 or 75 mg tablets. However, both are not in the HA drug formulary.

Source: www.fda.gov

Misoprostol Effective for Small Bowel Ulcers Associated With NSAIDs

Date: May 09, 2018

Aspirin and other non-steroidal anti-inflammatory drugs are commonly used drugs for reducing inflammation and relieving pain. However, the use of them can cause obscure gastrointestinal bleeding and even ulcers. There were studies that proved the effectiveness of misoprostol (synthetic analog of prostaglandin E1) in gastric and duodenal ulcers. However, none of them have focused on small bowel ulcers.

A double-blind, phase 3 trial was conducted in UK to investigate the potential effectiveness of misoprostol in healing small bowel ulcers (from 2016-2017). 104 patients aged 18 or above, with small bowel ulcers and concurrent NSAIDs consumption for at least 4 weeks, were recruited. They are randomly assigned (1:1) to receive 200 mcg oral misoprostol or placebo four times daily for 8 weeks. The primary outcome was the complete healing of small bowel ulcers and erosions. Other outcome includes safety.

Complete healing was observed in 54% and 17% of patients in the misoprostol and placebo groups respectively (36.7% difference, 95% CI: 19.5-53.9, p=0.0002). Adverse events occurred in 46% and 42% of patients in the misoprostol and placebo group respectively. The most common adverse events were abdominal pain, nausea or vomiting, and diarrhoea. 8% of patients in the misoprostol group had severe adverse events, compared with none in the placebo group. No serious adverse events were reported.

Misoprostol is effective for the treatment of small bowel ulcers and erosions in NSAID-users. However, the benefit needed to be balanced against the risk of side-effects.

Source: www.thelancet.com

FDA: Gilenya Approved for Multiple Sclerosis in Paediatric Patients

Date: May 11, 2018

According to U.S. Food and Drug Administration (FDA), Gilenya (fingolimod) was originally indicated for relapsing multiple sclerosis (MS) for adult in 2010. FDA has recently extended its approval on Gilenya to cover children and adolescents aged 10 years or above. It is the first approval from FDA on drug to treat MS in paediatric patients.

MS is a chronic, inflammatory and autoimmune disease of the central nervous system. It disrupts the connection between the CNS with other parts of the body. Despite treatment, the patients will relapse after the initial recovery period with worsening CNS function and emerging new symptoms.

Gilenya is patented by Novartis. A double-blind, randomized, multicentre, active-controlled clinical trial is conducted. Daily oral therapy of Gilenya was compared with weekly intra-

muscular therapy of interferon beta-1a in terms of safety and efficacy in paediatric patients (< 18 years old) with MS. Gilenya outperformed interferon beta-1a in terms of relapse-free duration (86% versus 46%). Meanwhile, the safety profile of Gilenya toward paediatric patients is similar to adult counterpart.

According to FDA, Gilenya must be dispensed with a patient Medication Guide, warning patients about the severe risk of lowering heart rate and severe infection.

For Hong Kong, Gilenya is currently registered in Hong Kong as 0.5 mg hard capsule and belongs to the category of "self-financed item with safety net coverage" in the HA drug formulary.

Source: www.fda.gov

Evaluation on the Potential Risk of Neural Tube Birth Defects with Dolutegravir

Date: May 18, 2018

FDA noticed the public that severe cases of neural tube defects involving the brain and spine had been reported in infants born to women taking dolutegravir to treat human immunodeficiency virus (HIV). Preliminary results from an ongoing observational study found that there was a higher risk for these defects in women who received dolutegravir at the time of becoming pregnant or early in the first trimester.

To date, there are no reported cases of babies born with neural tube defects to women starting dolutegravir later in pregnancy. Dolutegravir is an FDA-approved antiretroviral agent used in combination with other antiretroviral drugs to treat HIV.

Health care professionals should notify women of childbearing age about the potential risk of neural tube defects when a dolutegravir-containing regimen is used at the time of conception or early in pregnancy. It is also important to weigh the benefits and the risks of dolutegravir when prescribing to women of childbearing age. Reinforcement of message regarding the consistent use of effective birth control is needed if the decision is made to use dolutegravir in women of childbearing age. Alternatives should be considered otherwise.

Source: www.fda.gov

Potentially Fatal Blood Disorder of Oral Over-the-counter Benzocaine Product

Date: May 23, 2018

Recently FDA is warning that the over-the-counter oral drug products containing benzocaine should not be used to treat children 2 years and younger. These products have minimum or no benefits and carry serious risks for treatment of sore gums in infants due to teething. Benzocaine can lead to a condition in which the amount of oxygen carried in the blood is largely reduced, which is known as methemoglobinemia.

Methemoglobinemia is life-threatening. Due to the significant risk, FDA has urged manufacturers to stop marketing OTC oral drug products for treating teething in infants and children younger than 2 years.

In addition, FDA has suggested the manufacturers to add a warning about methemoglobinemia on the drug labelling of the benzocaine-containing products. Other measure includes adding contraindications to direct parents and caregivers not to use the product for teething and not to use in infants and children younger than 2 years. Health care professionals should warn patients of the possibility of methemoglobinemia and advise them about the signs and symptoms when recommending or prescribing local anaesthetic products of related compounds.

Source: www.fda.gov

EMA Restricts Use of Keytruda and Tecentriq in Bladder Cancer

Date: June 01, 2018

Early data from two clinical trials has shown reduction in survival with Keytruda (pembrolizumab) and Tecentriq (atezolizumab) when used as first-line treatments for urothelial cancer in patients with low levels of a protein called PD-L1. The data revealed that Keytruda and Tecentriq may not work with same efficiency as chemotherapy in this group of patients. EMA has suggested to restrict the use of these medicines as first-line treatment for urothelial cancer.

Keytruda and Tecentriq should now only be used for first-line treatment of urothelial cancer in patients with high levels of PD-L1. There are no changes to how these medicines should be used in patients with

urothelial cancer who have had chemotherapy or in patients with other cancers for which these medicines are approved.

In Hong Kong, Keytruda Solution for Injection 100mg/4ml (HK- 64228) and Keytruda Powder for Injection 50mg (HK- 64229) are registered. All three products are prescription-only medicines and are indicated for urothelial carcinoma. In view of the EMA announcement on the restriction, the matter will be discussed by the Registration Committee of the Pharmacy and Poisons Board.

Source: www.drugoffice.gov.hk

Sulfonylurea Safe and Effective for KCNJ11 Permanent Neonatal Diabetes

Date: June 04, 2018

KCNJ11 mutations cause neonatal diabetes due to impaired insulin secretion. After insulin therapy, the patients are transitioned to oral sulfonylureas. The initial glycaemia control for this population is good with the transition. However, the long term effect of sulfonylurea is unclear.

Therefore, a multicentre, international cohort study was carried out. 81 patients with KCNJ11 permanent neonatal diabetes were recruited from 5 laboratories in UK, Italy, Norway and France. They all had transferred to sulfonylurea before Nov 30, 2016, and were followed up for a median duration of 10.3 years. The primary outcomes were sulfonylurea failure, HbA1C and sulfonylurea at most recent follow-up. Associated neurological features were also examined.

93% of participants remained on sulfonylurea therapy alone. Excellent glycaemic control was at all time points - median HbA1c was 8.1% before transfer, 5.9% ($p < 0.0001$ vs pre-transfer) at 1 year, and 6.4% ($p < 0.0001$ vs year 1) at most recent follow-up. The median sulfonylurea dose at 1 year was 0.30 mg/kg per day and at most recent follow-up visit was 0.23 mg/kg per day ($p = 0.03$). 9% patients had microvascular complications and has taken insulin longer than those without complications (median age at transfer to sulfonylureas 20.5 years vs 4.1 years; $p = 0.0005$). After long-term therapy with sulfonylureas, CNS features were seen in 64% of patients.

High-dose sulfonylurea therapy is safe and highly effective in patients with KCNJ11 permanent diabetes.

Source: www.thelancet.com

Another Career Option from Clinical Work – Interview with Vincent Wong

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INTRODUCTION

Working in hospitals or community pharmacies may be a dream career path to many pharmacy graduates. Vincent Wong, a registered pharmacist in Hong Kong, chose a different path after his graduation and tells a different story with his choice of working in the pharmaceutical industry.



Figure 1. Vincent Wong

BACKGROUND

Vincent received his Bachelor degree of Pharmacy from The Chinese University of Hong Kong (CUHK). Upon graduation, Vincent started his career in 2015 in GlaxoSmithKline (GSK), a multi-national pharmaceutical corporation, and is currently the Regulatory Affairs Executive in the GSK Consumer Health Division.

PJ: Why would you decide to work in Regulatory Affairs (RA) department?

Vincent: I worked as an Industrial Placement in GSK before, when RA was integrated as part of the Medical Affairs (MA) department. The working experience allowed me to have a glimpse on the daily operation of RA department and assist in different work of RA, with preparing submission dossiers to the Department of Health (DH) being one of those. Compared with the job nature in hospitals or community pharmacies, I found that the work in RA is much more challenging. The daily work of RA involves lots of communication with different stakeholders and related parties, including pharmacists in DH, and colleagues from other departments and global level, which needs many negotiations to ensure all things go smoothly.

PJ: What is the role of Regulatory Affairs (RA) in pharmaceutical industry? What does that mean to you?

Vincent: RA department is mainly responsible for drug registration. RA submits product dossiers to DH and the efficacy, safety and quality of the drug are assessed. Without registration, the drug cannot be put into market and patient access is impossible. To me, RA serves as the “initiator” to bring new therapeutic options to the patients locally. RA is involved in the entire product life cycle and manages risks and incidents including product recalls. We communicate with DH closely to ensure patient safety and product with good quality is supplied to the patients.

With GSK being a member of the Hong Kong Association of the Pharmaceutical Industry (HKAPI), we also work with other pharmaceutical companies and DH to revise current DH guidelines and implement new policies related to pharmaceutical products. We drive changes in the industry, hoping to improve the whole public health system.



Figure 2. Vincent gave a workshop on the Role of RA at The University of Hong Kong (HKU).

PJ: Could you tell us more about your daily duties, as a Regulatory Affairs Executive?

Vincent: The first thing on my working list is unquestionably drug registration. I usually deal with product changes, such as updating the information in product inserts. On top of that, I have to communicate with colleagues from global team regarding the change in products, for instance, the country

requirement, documents required and discuss with commercial colleagues for the submission as well as the approval timeline of the drug registration process. Apart from drug registration, my daily duties also include promotional material review, in which we make sure the promotional materials do not infringe the Undesirable Medical Advertisements Ordinance (UMAO). Optimizing internal processes, updating standard operating procedures (SOP), conformance check and providing training to colleagues are also something that I work on in my daily routine.



Figure 3. Besides working, Vincent actively participates in different sports activities to have a work-life balance.

PJ: How does your pharmacy knowledge help in your daily work in pharmaceutical industry?

Vincent: The knowledge of pharmacy laws and regulations that I learnt in pharmacy school helps me the most. Just reading the law statements, we may not be able to understand the original intention of the legislation. In lectures, however, we have learnt the spirit of the legislation behind. For example, the particulars in a transaction record such as delivery address and batch number are essential. This allows us to trace the products in a product recall. The basic drug knowledge I gained in university has also enabled me to understand the technical terms and data in the product dossiers, such as specifications, stability and pharmacokinetics data, which are important in DH dossier submission. What I learnt in pharmacy school does help me put the scientific knowledge into real practice.

The 4-year university study has not only equipped me with drug knowledge, but also has nurtured me to be a more careful and detail-minded person. This character is vital in RA work. Any mistakes in the dossiers submitted to DH may become a barrier in processing the drug registration application, delaying the whole approval process for several months.

PJ: Are there any challenges in your work?

Vincent: Pharmaceutical industry is an always changing world, with regulations and guidelines keep being updated. As

a RA staff member, we have to keep an eye on the changes and to react fast to cope with the dynamic environment. I remembered that there was a change of “poison logo” in 2016 and we needed to change the product packaging as per the timeline imposed by DH. Time was limited and we had to work closely with other teams to carry out the change smoothly and efficiently.

Another challenge that I encountered is communication. As I have mentioned before, we have to communicate with different parties, including internal stakeholders, healthcare professionals, DH and Hospital Authority (HA), which is a difficult task to me as well. Many negotiations and efforts are required to come up with a solution that fulfils the expectations of all parties.

PJ: What advice will you give to the students who are interested to develop their career in the pharmaceutical industry?

Vincent: The most important thing is to be open-minded. The main focus of current pharmacy curriculum is clinical knowledge, which you may not be able to apply directly on the work in pharmaceutical industry. Therefore, you may need to re-position yourself and adjust your expectations. More importantly, be humble and grab every chance to get acquainted with the commercial world.



Figure 4. Vincent took part in the Dragon Boat Race organized by The Hong Kong Medical Association to build up team spirit with colleagues.

Moreover, keep yourself updated with new clinical knowledge, policies and regulations which can be related to your career in the industry. Honing your soft skills, especially communication skills, will put you at an advantageous place in your career as well.

CONCLUSION

The story of Vincent has demonstrated the unlimited possibility of career development in the pharmaceutical industry. It is a high time for us to think out of the box to explore the role of pharmacist in the pharmaceutical industry.

Author's background

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Review of Newly Registered Anticoagulants and a Reversal Agent for Dabigatran: Bivalirudin, Edoxaban and Idarucizumab

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ABSTRACT

New medications which affect the coagulation cascade have been added to the Hong Kong market recently. With the addition of these medications, it is important to understand each agent's role in therapy. We conducted a literature review of 2 newer anti-coagulants Bivalirubin and Edoxaban as well as reversal agent Idarucizumab. With each medication, we focused on their indication, mechanism of action, notable adverse effects and unique characteristics as well as a comparative analysis of each against the long-standing agents within its therapeutic class. Bivalirudin may be highly useful in the management of heparin-induced thrombocytopenia as other agents used in this disease state are not available in Hong Kong. Edoxaban is likely to have limited usefulness as it has unique stipulations that limit its convenience including that patients must have a creatinine clearance of less than or equal to 95mL/min and that parenteral anticoagulants must be used for 5 to 10 days prior. Idarucizumab is the first approved reversal agent for the direct oral anticoagulants, specifically dabigatran. Idarucizumab will have a role in therapy, but should be restricted to life-threatening bleeds in patients taking dabigatran due to significant cost concerns. This article provides a summary of the major clinical points for each agent to allow pharmacists to better select and optimize drug use in patients.

Keywords: Anticoagulants, Bivalirubin, Edoxaban, Idarucizumab

INTRODUCTION

Warfarin and heparin have been the major anticoagulants prescribed in the past. However, warfarin and heparin have many limitations leaving a large gap in therapeutic management of patients. The shortcomings of warfarin and heparin include many drug-drug interactions, a narrow therapeutic window, less predictable pharmacological effects, inconvenient dosing and frequent monitoring. Newer anticoagulants provide us with more options to avoid these limitations. In this article, we focus on bivalirudin and edoxaban, which are new anticoagulants in Hong Kong; and idarucizumab, which is a reversal agent for dabigatran. All three of which were registered in Hong

Kong between April and October 2016. Their indications, pharmacology, side effects and efficacy are presented below.

BIVALIRUDIN (Angiox[®])

Approved Indication

Bivalirudin is only available in Hong Kong with the brand name Angiox[®]. Although it is new to Hong Kong, it has been used in other countries since the turn of the century. The legal classification of the drug in Hong Kong is P1S1S3.⁽¹⁾ Bivalirudin is the first parenteral direct thrombin inhibitor registered in Hong Kong as desirudin and argatroban are not currently registered. The use of bivalirudin is expected to be limited in Hong Kong largely due to cost. The average wholesale cost in the USA for the 250 mg vial branded product is over \$9000 HKD, and approximately \$4000 HKD for the generic product.⁽²⁾

Bivalirudin is approved by the Food and Drug Administration (FDA) and European Medicines Association (EMA) for use in percutaneous coronary intervention (PCI), where it is used in conjunction with aspirin and a Glycoprotein IIb/IIIa inhibitor. It is also used in conjunction with aspirin in patients with unstable angina undergoing percutaneous transluminal coronary angioplasty (PTCA). Furthermore, bivalirudin may be used as an anticoagulant for patients with or at risk of heparin-induced thrombocytopenia (HIT).⁽²⁻⁴⁾

Classification and Mechanism

Bivalirudin is a specific and reversible direct thrombin inhibitor. It works by binding the catalytic and anionic exosite of both circulating and clot-bound thrombin. Catalytic binding site occupation functionally inhibits coagulation effects by preventing thrombin-mediated cleavage of fibrinogen to fibrin monomers, and activation of factors V, VIII, and XIII. It also shows linear dose- and concentration-dependent prolongation of activated clotting time (ACT), activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin time (TT).⁽²⁾

Evidence from Clinical Studies

Percutaneous Coronary Intervention/ Percutaneous transluminal coronary angioplasty (PCI/PTCA):

The approved indication of bivalirudin in PCI anticoagulation is supported by several clinical trials. In older studies such as the REPLACE-2 randomized trial (2003) with 6,010 patients and the bivalirudin Angioplasty Study (2001) with 4,312 patients, comparison of bivalirudin versus heparin with or without GpIIb/IIIa blockade in either arm was statistically non-inferior with regard to the suppression of acute ischemic end points and is associated with less bleeding.^(5,6) More recently, the updated results from the ISAR-REACT 4 trial (2013) suggested that in patients with NSTEMI undergoing PCI, abciximab with heparin versus bivalirudin provided comparable outcomes for one year, although bivalirudin had a lower rate of bleeding at 30 days.⁽⁷⁾ The HORIZONS-AMI trial (2011) with 3,602 patients and the EUROMAX trial (2013) with 2,218 patients showed that the incidence of bleeding was significantly less and the rates of thrombosis were significantly higher in patients treated with bivalirudin versus patients treated with heparin plus a GpIIb/IIIa inhibitor.^(8,9)

Bivalirudin may have a role in the anticoagulation management of patients undergoing a PCI/PTCA, particularly in patients at a high risk of bleeding. The question remains whether the less bleeding and possibly more thrombosis seen with bivalirudin use is worth the drastic difference in price compared to heparin. Three pharmacoeconomic studies were completed overseas comparing bivalirudin versus heparin and they all concluded that bivalirudin was cost effective.⁽¹⁰⁻¹²⁾ However, a local study would be highly beneficial in determining if these clinical and economic outcomes hold true in the local Hong Kong population.

Heparin Induced Thrombocytopenia:

Patients that develop HIT have further increased risk of thrombosis due to platelet activation and release of procoagulant, platelet-derived microparticles.⁽¹³⁾ This is in addition to the underlying indication for which they were originally receiving heparin. Therefore, discontinuation of anticoagulation is not suitable. This statement is in line with a strong recommendation (Grade 1C) from the 2012 CHEST guidelines.⁽¹³⁾ Despite this recommendation, some patients with a documented HIT proceed untreated without an anticoagulant for their underlying condition. With the registration of bivalirudin, the health care team now have two available options for patients with HIT: fondaparinux (Arixtra[®]) or bivalirudin. The 2012 Chest guidelines for HIT recommend the use of argatroban or danaparoid as there is more available literature supporting their use, but neither of which are registered in Hong Kong. However, bivalirudin is an alternative as it is the same class of drug as argatroban. The CHEST guidelines recommend bivalirudin as the first-line therapy for the subset of patients with HIT who need to undergo urgent cardiac surgery.⁽¹²⁾ Bivalirudin can be used in patients with HIT as the other preferred agents are not registered in Hong Kong. Fondaparinux may be used in pregnancy for the management of HIT when danaparoid is unavailable.⁽¹³⁾ Bivalirudin may also be considered in pregnancy as it is listed as pregnancy category B.⁽²⁾

Adverse Effects

Bivalirudin has less bleeding potential when compared to heparin, but may still cause some side effects. However, none

of the side effects are more frequent when compared to heparin. The only adverse effect that appears to be more common in bivalirudin than heparin is the thrombosis rate. Other adverse effects with an incidence rate greater than 0.5% are headache, thrombocytopenia, and fever.⁽⁴⁾ These adverse effects may be symptoms of the disease rather than the adverse effects of bivalirudin or heparin.

Notable Medication Characteristics

Bivalirudin has a short half-life of 25 minutes.⁽²⁾ Therefore, its anticoagulant effect wears off quickly and accounts for the reduced bleeding risk after PCI, making it an adequate alternative to low molecular weight heparins (LMWH) when used in PCI.⁽⁵⁾ In addition, it requires a simpler pharmacodynamic monitoring and the dose is based on weight. **(Table 1)** The initial dose is 0.75 mg/kg intravenous (IV) bolus immediately prior to procedure, followed immediately by 1.75 mg/kg/hour for the duration of procedure and optionally up to 20 hours after.⁽²⁾ Renal adjustment is required for patients with a creatinine clearance of less than 30 mL/min. A reduction of infusion rate to 1 mg/kg/hour should be considered when used in PCI.⁽²⁾

EDOXABAN (Lixiana[®])

Approved Indication

Edoxaban was newly registered under the brand name Lixiana[®] in Hong Kong in May 2016.⁽¹⁾ It is available in tablet forms with the strengths of 15, 30 and 60mg.⁽¹⁾ The legal classification for edoxaban in Hong Kong is P1S1S3.⁽¹⁾ Edoxaban is approved for the prevention of stroke and systemic embolism in adult patients with non-valvular atrial fibrillation (NVAf), and treatment of deep vein thrombosis and pulmonary embolism as approved by both the FDA⁽¹⁴⁾ and EMA.⁽¹⁵⁾ Although it lacks some indications that the other factor Xa inhibitors possess **(Table 2)**, clinical trials are currently being conducted to identify potential new indications.⁽¹⁶⁾

Classification and Mechanism

Edoxaban is an oral anticoagulant and the third factor Xa inhibitor approved in Hong Kong. Edoxaban inhibits free factor Xa and thus prothrombinase activity, which is essential in the clotting cascade. This prevents the stepwise amplification of coagulating factors and thrombin generation, thereby reducing the thrombin-induced platelet aggregation.⁽¹⁷⁾

Evidence from Clinical Studies

Clinical trials have not compared edoxaban directly with the other factor Xa inhibitors. Edoxaban is a potential alternative to warfarin for the prevention of stroke and systemic embolism in patients with NVAf. ENGAGE AF-TIMI⁽¹⁸⁾ is a phase 3 trial which demonstrated that both 30mg and 60mg once daily edoxaban regimens were non-inferior to warfarin. Edoxaban had a significantly lower risk of the primary outcome (death from any cause, stroke or systemic embolic event) than warfarin. The Hokusai-VTE study⁽¹⁹⁾ with 8,292 patients was conducted to compare the safety and efficacy of edoxaban

NOACs	Bivalirudin ⁽²⁾	Dabigatran ⁽³⁰⁾	Edoxaban ⁽¹⁷⁾	Rivaroxaban ⁽²¹⁾	Apixaban ⁽²²⁾
Brand Name	Angiox [®]	Pradaxa [®]	Lixiana [®]	Xarelto [®]	Eliquis [®]
Mechanism	Thrombin (factor II) inhibitor	Thrombin (factor II) inhibitor	Factor Xa inhibitor	Factor Xa inhibitor	Factor Xa inhibitor
Bioavailability	Not Applicable	3%-7%	>62%	10 mg: 80 ~100% 20mg: 66% (fasting)	50%
Time to Reach Peak Level (T_{max})	IV bolus: 15min SC: 2hr	1 hour	1-2 hour	2-4 hours	3-4 hours
Elimination Half-life (t_{1/2})	25 minutes	12-17hour	5-9hour	Healthy: 5-9 hours Elderly: 11-13 hours	10-14hour
Effect of Food	None	None	None	20 mg, take with food	None
Renal Clearance	20%	80%	66% (36% unchanged, 30% inactive metabolite)	36%	25%
Metabolism*	Blood Proteases	Glucuronidation	Minor CYP3A4, not clinically significant	CYP3A4/5 and CYP2J2	Mainly CYP3A4/5
Pharmacodynamic Monitoring	Activated clotting time	Ecarin clotting time, thrombin time, aPTT and ACT	Direct Xa activity, PTT	Direct Xa activity, PTT	Direct Xa activity, PTT
Available Strengths in Hong Kong	250 mg vial	75 mg, 110 mg, 150 mg capsule	15 mg, 30 mg, 60 mg tablet	10 mg, 15 mg, 20 mg tablet	2.5 mg, 5 mg tablet
Reversal Agent	None, Short t _{1/2}	Idarucizumab	Andexanet alfa*	Andexanet alfa*	Andexanet alfa*
Dosing Regimen	Continuous infusion	Twice daily	Daily	Daily	Twice daily

*Not registered in Hong Kong nor approved by the FDA as of April 2018

All novel oral anticoagulants are P-glycoprotein substrates

NOACs: New Oral Anticoagulants

Indications as of April 2018	Rivaroxaban	Apixaban	Edoxaban
Reduction in risk of stroke and systemic embolism in NVAf	X	X	X
Treatment of DVT and PE	X	X	X
Secondary risk reduction of DVT/PE in previously treated patients	X	X	
Prevention of DVT/PE in patients undergoing hip or knee replacement	X	X	

60 mg once daily treatment to standard therapy of warfarin, after initial treatment with heparin in preventing the recurrence of venous thromboembolism (VTE). This study showed that edoxaban regimen was non-inferior to warfarin in the primary endpoint of recurrent VTE (3.2% vs 3.5%), with a significant decrease in clinically relevant bleeding risk (8.5% in edoxaban group and 10.3% in warfarin group).

Adverse Effects

The most frequent adverse effect is hemorrhage, which can occur at many different anatomical sites and result in anemia. Other common side effects include skin rash and abnormal hepatic function tests, but the incidence rates of these side effects were not significantly different than in patients receiving warfarin.^(20,21) The boxed warning includes the increase in risk of ischemic events from premature discontinuation of edoxaban and spinal/epidural hematoma.⁽¹⁷⁾ This boxed warning applies to all factor Xa inhibitors.^(21,22)

Notable Medication Characteristics

The use and characteristics of edoxaban are similar to the other medications in the same class. The once-daily dosing of edoxaban provides an advantage over apixaban and dabigatran for patients with poor compliance in twice-daily dosing. Edoxaban is dosed without regard to meals and is

only metabolized by Cytochrome P450 3A4 (CYP3A4) to a minor extent, resulting in fewer clinically significant drug-drug interactions compared to the other factor Xa inhibitors.⁽²³⁾ **(Table 1)** Unlike the other two oral factor Xa inhibitors, edoxaban has some unique caveats to consider prior to recommending its use in patients. When used for NVAf, edoxaban should be avoided when creatinine clearance is greater than 95 mL/min because of the increased risk of ischemic stroke compared with use of warfarin. Secondly, patients with DVT should be treated with parenteral anticoagulants for 5 to 10 days prior to the commencement of edoxaban therapy, but this is not required for apixaban and rivaroxaban.⁽²³⁾ In addition to the precautions carried by the other factor Xa inhibitors, there is also the precaution for the use of edoxaban in moderate to severe mitral stenosis in which the other factor Xa inhibitor do not have this precaution.^(17,21,22) Patients with weight not more than 60 kg or patient with concomitant therapy with specific P-gp inhibitors should receive edoxaban 30 mg once daily. The dose should be reduced from 60 mg to 30 mg for patients with a creatinine clearance of 15-50 ml/min. The 15 mg tablets should only be used in the switching between edoxaban and warfarin. If surgery or other interventions are required, edoxaban should be discontinued at least 24 hours prior to the procedure to reduce the risk of bleeding.

IDARUCIZUMAB (Praxbind[®])

Approved Indication

Idarucizumab (Praxbind[®]) is available in a 2.5 g/ 60 mL solution for injection/ infusion that requires refrigeration.⁽²⁴⁾ It is a specific reversal agent for dabigatran with legal classification of P1S1S3 in Hong Kong.⁽¹⁾ It is indicated, with approval from the FDA⁽²⁵⁾ and EMA⁽²⁶⁾, in patients treated with dabigatran when rapid reversal of the anticoagulant effects is required for emergency surgery/ urgent procedures or in life-threatening or uncontrolled bleeding.

Table 3. Comparison of Different Hemorrhage Reversal Agents

Reversal Agents	Idarucizumab ⁽²⁴⁾	Beriplex ⁽³¹⁾	Fresh Frozen Plasma ⁽³³⁾	Novoseven ⁽³²⁾
Pharmacologic Classification	Humanized Monoclonal Antibody	Prothrombin Complex Concentrate	Blood Product	Factor VIIa (Recombinant)
Time of Onset	Within minutes	Rapid; INR decline < 10 minutes	N/A	10-20 min
Specificity	Dabigatran only	Vitamin K Antagonist	None	No specificity
Cost (HKD) ^(34,35)	> \$26,000 per dose	\$12,736-25,472	~\$1962	\$17390

INR: International Normalized Ratio

Classification and Mechanism

Idarucizumab is a humanized monoclonal antibody fragment (Fab) that binds to dabigatran with a very high affinity, that is approximately 300-fold more potent than the binding affinity of dabigatran to thrombin.^(25,26) This high affinity for dabigatran means any unbound dabigatran will preferentially bind to idarucizumab.⁽²⁶⁾ This idarucizumab-dabigatran complex, with a long half-life, is characterized by a rapid on-rate and extremely slow off-rate, resulting in a very stable and practically irreversible binding.⁽²⁶⁾ Therefore, it can potently and specifically bind to dabigatran and its metabolites, reversing the anticoagulant effect.

Evidence from Clinical Studies

The phase 3 RE-VERSE AD⁽²⁷⁾ single-arm study is currently ongoing to investigate the safety and efficacy of 5 grams of idarucizumab in patients who presented with dabigatran-related life-threatening or uncontrolled bleeding (Group A) or who required emergency surgery or urgent procedures (Group B). An interim analysis included data from 123 patients, of which 90 patients received idarucizumab (51 from Group A and 39 from Group B). More than 89% of patients, in both Group A and B, achieved complete reversal of anticoagulant effect of dabigatran as measured by dilute thrombin time or ecarin clotting time) in the first 4 hours after the administration of 5 grams of idarucizumab. Reversal of pharmacodynamic effects were evident immediately after administration.⁽¹⁸⁾ Results for Group A and B were similar. 20% of patients required a second dose of idarucizumab. The study completed in 2016, but publication of further results is still pending.

Frequent Side Effects

Serious adverse effects for idarucizumab include thromboembolic risk and hypersensitivity reactions (pyrexia, bronchospasm, hyperventilation, rash and pruritus).⁽²⁶⁾ Other adverse effects reported in greater than 5% of patients are hypokalemia, constipation, pneumonia and delirium.^(25,26) The reversal of dabigatran therapy may expose patients to a thrombotic risk of their underlying diseases.⁽²⁵⁾ To minimize the risk, anticoagulant therapy should be resumed as soon as medically appropriate.⁽²⁵⁾

Notable Medication Characteristics

Idarucizumab is the only commercially available agent specifically for the reversal of anticoagulant effect of dabigatran. Unlike the other reversal methods like prothrombin complexes or platelet infusions, idarucizumab does not have

prothrombotic activity as it does not replenish new coagulating factors.⁽²⁹⁾ Unlike blood product infusions, it does not cause rejection or volume expansion. Thus, it facilitates the resumption of anticoagulant therapy after the reversal treatment. Its specificity also allows it to be used simultaneously with other supportive measures in the management of hemorrhage. It has no impact on the effect of other anticoagulant or antithrombotic therapies. **(Table 3)** It is given as two consecutive infusions over 5-10 minutes each, or as a bolus injection.⁽²⁶⁾ No dose adjustment is required in patients with renal impairment.⁽²⁶⁾

CONCLUSION

Of the three newly registered cardiovascular drugs in Hong Kong, edoxaban and bivalirudin are two new anticoagulants, while idarucizumab is a specific antidote for dabigatran. Bivalirudin is the first in its class to be registered in Hong Kong and can fill the gap in the current clinical practice in Hong Kong. The benefits of edoxaban are not unique when compared to other new oral anticoagulants. Advantages such as the once daily dosing is also the same dosing interval for rivaroxaban and the less drug-drug interactions is also seen with dabigatran. The less bleeding risk compared to warfarin is not unique either as the other new oral anticoagulants have data showing less bleeding when compared to warfarin. Lastly, it is expected that the development of idarucizumab can allay the concern of no reversal agents and can increase the utilization of dabigatran. However, the choice of medication is also subject to financial consideration, disease status and patients' preference. Many upcoming changes are expected in the area of anticoagulation, as anticoagulation is a huge financial opportunity for pharmaceutical companies. Therefore many resources are invested in research and development in this field of therapeutics.

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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.)

1. Which therapy is recommended in the 2012 Chest guidelines for patients with heparin-induced thrombocytopenia (HIT) who undergo urgent cardiac surgery?

- A. Argatroban
- B. Fondaparinux
- C. Bivalirudin
- D. Any low molecular weight heparin

2. All of the followings are benefits of bivalirudin EXCEPT:

- A. It is the first of its class (parenteral direct thrombin inhibitor) available in Hong Kong
- B. It is associated with less bleeding compared to heparin in patients undergo PCI
- C. Bivalirudin has a short half-life which allows the effect to wear off quickly in case of a bleed or overdose
- D. Bivalirudin is superior in preventing thrombosis compared to unfractionated heparin in patients undergoing PCI

3. The most common side effects associated with edoxaban include:

- A. Nausea, bleeding, and diarrhea
- B. Anemia and bleeding
- C. Upper gastrointestinal bleed and hemorrhagic stroke
- D. Abnormal liver function test and heartburn

4. Edoxaban belongs to which of the following class of medications?

- A. Direct thrombin inhibitor
- B. Factor Xa inhibitor
- C. Vitamin K antagonist
- D. P2Y12 inhibitor

5. For prevention of stroke in patients with NVAf, edoxaban may be utilized for patients with which range of creatinine clearance (CrCl) values?

- A. CrCl > 95 mL/min
- B. CrCl < 15 mL/min
- C. CrCl 15 to 95 mL/min
- D. CrCl 35 to 105 mL/min

Please refer to the following case for questions 6-8.

J.T. is a 62-year-old Chinese male who was admitted to the hospital due to embolic stroke. The patient weighs 62 kg and has no known drug allergies. His past medication history is significant for type 2 diabetes mellitus, atrial fibrillation, hypertension, and constipation. He is currently on carvedilol 12.5 mg twice daily, hydrochlorothiazide



2 CE Units

Review of Newly Registered Anticoagulants and a Reversal Agent for Dabigatran: Bivalirudin, Edoxaban and Idarucizumab

25 mg daily, metformin 1000 mg twice daily with meals, and aspirin 160 mg daily. He is using aspirin for anticoagulation because he did not want to use warfarin. His INR was not stable and he disliked obtaining frequent INR checks. He is soon to be discharged from the hospital, and his physician is asking you about the use of edoxaban.

6. What is the appropriate dose of edoxaban for J.T. if his CrCl is 57 mL/min?

- A. 15 mg daily
- B. 30 mg daily
- C. 60 mg daily
- D. 90 mg daily

7. How should J.T. be advised in proper administration of edoxaban?

- A. Once daily on an empty stomach
- B. In divided doses twice daily with meals
- C. Once daily with the evening meal
- D. Once daily with or without food

8. J.T. is going to have a colonoscopy and hemorrhoidectomy scheduled in 10 days from now. When should he discontinue his edoxaban?

- A. At least 24 hours prior to hemorrhoidectomy
- B. Discontinuation is unnecessary, continue therapy as usual
- C. At least 7 days prior to hemorrhoidectomy
- D. He should be transitioned to enoxaparin for 3-5 days prior to hemorrhoidectomy.

9. Idarucizumab can be used for reversal of which of the following agents?

- I. Dabigatran
 - II. Bivalirudin
 - III. Apixaban
 - IV. Edoxaban
- A. All of the above
 - B. I, II, IV
 - C. I and III
 - D. I only

10. Which of the following is a common adverse effect of idarucizumab?

- A. Hyperkalemia
- B. Urinary tract infection
- C. Rash
- D. Paresthesia

Answers will be released in the next issue of HKPJ.

CE Questions Answer for 251(D&T)

Understanding Adverse Drug Reactions and Drug Allergies: a Brief Overview

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

Evaluation of Bioactive Chemicals in Rhododendri Species by Principle Chemical Analysis

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ABSTRACT

Methods of both thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) have been developed in our lab for identification of *Rhododendron dauricum* L. (Ericaceae). These methods could be used to authenticate and evaluate the chemical profiles of this species from other Rhododendri species. The TLC method offers brief fingerprint patterns of different Rhododendri species. It supports the detailed observation in HPLC. The HPLC method has been validated to demonstrate its reproducibility. When principle chemical analysis was applied to eight species of the Rhododendron genus, the results indicate that they are truly different from each other as their chemical profiles are different from each other. The result of both HPLC and TLC study reveals that *Rhododendron dauricum* L. (Ericaceae) is different from other species of the Rhododendron genus. In conclusion, the present approach is rapid and could be used for the quality assurance of Rhododendron species.

Keywords: *Rhododendron* genus, *Rhododendron dauricum* L., chemical profiles, TLC, HPLC, principle chemical analysis, authentication, farrerol,

INTRODUCTION

Rhododendron is one of the largest genera in the family of Ericaceae, which is subjected to much ongoing taxonomic debate because they look alike. So far, about 1,025 species have been identified and they distribute from the northern temperate zone, throughout tropical Southeast Asia, to northeastern Australia. In China, there are 571 species classified in 6 subgenera; of which 405 species are endemic. Apart from Xinjiang and Ningxia, Rhododendrons have been found in all other provinces.⁽¹⁾

Rhododendri Daurici Folium, which is the dried leaf of *Rhododendron dauricum* L. (Ericaceae), is a traditional Chinese Medicine. It was first described as a herbal medicine by the name of "manshanhong" in Handbook of Chinese Herbal Medicine Commonly Used in Northeast China and was first included in the Chinese Pharmacopoeia (2005) as an official herbal medicine. It has been used by Northeastern Chinese for years as antitussive and expectorant. However, this

herbal is easily confused with other species.^(2,3) It is hard to authenticate it simply based on morphological features of the dried leaves as they all look alike in the family of Rhododendrons (Ericaceae).

In this study, we applied HPTLC and HPLC method to find out the feasibility of identifying the Rhododendri Daurici Folium from other confusing species. The methods we have developed were found to be reliable; it was validated and confirmed that this approach could be used for the identification of *Rhododendron dauricum* L.

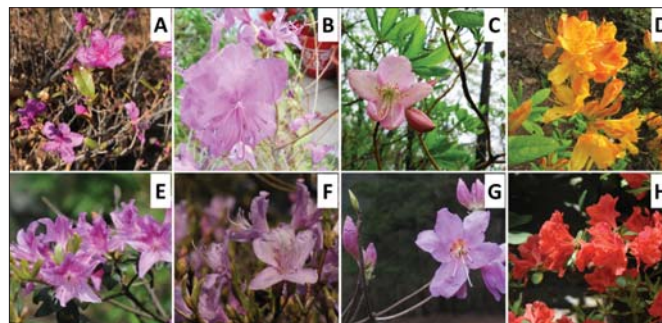


Figure 1. Photograph of 8 species of the Rhododendri genus. (A) *Rhododendron dauricum* L.; (B) *Rhododendron mucronulatum* Turcz.; (C) *Rhododendron schlippenbachii* Maxim.; (D) *Rhododendron molle* (Blume) G. Don; (E) *Rhododendron farrerae* Tate ex Sweet; (F) *Rhododendron mariesii* Hemsl.; (G) *Rhododendron huadingense* B. Y. Ding & Y. Y. Fang; (H) *Rhododendron simsii* Planch.

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of eight Rhododendron species were collected from different location of China. Except the *Rhododendron dauricum* L. (MSH), of which ten batches of leaves were collected from different sources and were authenticated by Dr. Zhifeng Zhang; one to three batches of other seven species were collected and authenticated by Dr. Lehua Zhang. **Table 1** shows the detail information of each sample; MSH was collected from various origins in Northern China while the others from one or two site.

All air dried leaves were cut into thin pieces and ground into powder using a blender to avoid sample bias. The powder

Table 1. Sample information of eight species of the *Rhododendri* genus used in this study.

Subgens	section	Species	Sample ID	Source
<i>Rhododendron</i> subgen. <i>Rhodorastrum</i> 迎紅杜鵑亞屬	/	<i>Rhododendron dauricum</i> L. 興安杜鵑 (MSH)	MSH001	Tonghua, Jilin
			MSH002	Ji'an, Jilin
			MSH007	Zhalantun, Neimenggu
			MSH008	Zhalantun, Neimenggu
			MSH009	Daxing'anling, Heilongjiang
			MSH010	Daxing'anling, Heilongjiang
			MSH011	Jiamusi, Heilongjiang
			MSH012	Tonghua, Jilin
			MSH013	Siping, Jilin
			MSH014	Zhalantun, Neimenggu
		<i>Rhododendron mucronulatum</i> Turcz. 迎紅杜鵑 (YHDJ)	YHDJ001	Benxi, Liaoning
			YHDJ002	Dandong, Liaoning
			YHDJ003	Dandong, Liaoning
			YHDJ004	Dandong, Liaoning
<i>Rhododendron</i> subgen. <i>Tsutsusi</i> (Sweet) Pojarkova 羊躑躅亞屬	<i>Rhododendron</i> sect. <i>Pentanthera</i> 五花藥組	<i>Rhododendron molle</i> (Blume) G. Don 羊躑躅 (YZZ)	YZZ001	Lushan, Jiangxi
	<i>Rhododendron</i> sect. <i>Sciadorhodon</i> 十花藥組	<i>Rhododendron schlippenbachii</i> Maxim. 大字杜鵑 (DZDJ)	DZDJ001	Dandong, Liaoning
			DZDJ002	Dandong, Liaoning
<i>Rhododendron</i> subgen. <i>Tsutsusi</i> (Sweet) Pojarkova 映山紅亞屬	<i>Rhododendron</i> sect. <i>Brachycalyx</i> Sweet 輪生菜組	<i>Rhododendron farrerae</i> Tate ex Sweet 丁香杜鵑 (DXDJ)	DXDJ001	Dawutong Mountain, Shenzhen
			DXDJ002	Xiaowutong Mountain, Shenzhen
			DXDJ003	Lushan, Jiangxi
	<i>Rhododendron</i> sect. <i>Tsutsusi</i> 映山紅組	<i>Rhododendron mariesii</i> Hemsl. 守城滿山紅 (SCMSH)	SCMSH001	Lushan, Jiangxi
		<i>Rhododendron huadingense</i> B. Y. Ding & Y. Y. Fang 華頂杜鵑 (HDDJ)	HDDJ001	Lushan, Jiangxi
		<i>Rhododendron simsii</i> Planch. 杜鵑 (映山紅) (YSH)	YSH001	Lushan, Jiangxi

obtained by the blender were passed through a 50 mesh filter and the particle size of powder was around $355 \pm 15 \mu\text{m}$. Powder of leaves (1 g) was extracted twice with 70% methanol by sonication on ice for 15 min. Power output of the sonicator was 100 kHz. After filtration, the methanol extracts were combined and adjusted to the same volume before it was used for analysis.

TLC Identification Method

An unpublished TLC method had been developed by us for the identification of *Rhododendron dauricum* L. using two biomarkers; namely, hyperoside and farrerol; the chemical structure of these two markers are shown in **Figure 2**. Sample was extracted with 70% methanol and the extract was spotted onto a silica plate by a TLC system (CAMAG Linomat 5 semi-automatic sampler with Reprostar 3 video-documentation system). These two bio-markers were well separated with a developing solvent composing methanol: tetrahydrofuran: waters: formic acid in a ratio of 3.5:1.5:2.5:0.3 (v/v). Bands became visualized after spraying with 1% aluminum chloride in ethanol, and observed under UV light at 366 nm.

HPLC Identification Method

HPLC method had also been developed by us for the analysis of six selected biomarkers, including hyperoside and farrerol, of *Rhododendron dauricum* L. Sample was extracted with 70% methanol and biomarkers in the extract were well resolved using a Zorbax SB-C18 column at 40°C , with a binary gradient mobile phase composing 0.2% phosphoric acid and methanol, and detected at wavelength 310 nm by Agilent 1260

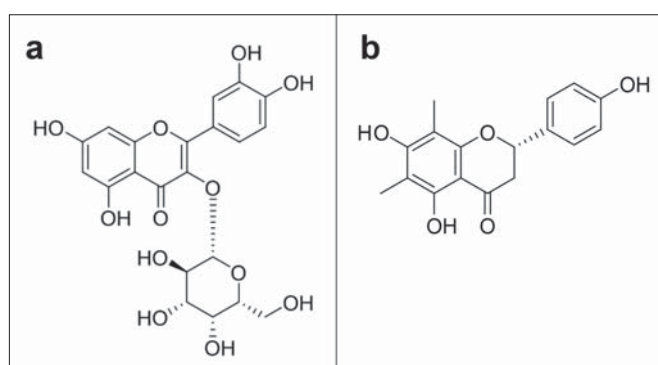


Figure 2. Structure of the two bio-marker in *Rhododendron dauricum* L. (a) hyperoside, (b) farrerol.

HPLC system. The HPLC method has been validated to test the reproducibility of fingerprints.

Statistical Analysis

All data were presented as mean from at least three independent experiments. Principal Chemical Analysis (PCA) conducted based on the correlation matrix was performed using the SIMCA 14 (MKS Instruments Pte Ltd., Singapore) software package for Windows.

RESULTS AND DISCUSSION

Species Comparison by TLC

A total of eight different species in the *Rhododendron* genus were undergone TLC analysis. Each of the *Rhododendron*

species has its unique chromatographic pattern under the same TLC condition (**Figure 3**).

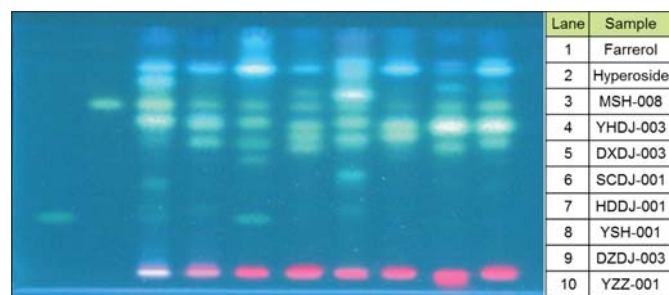


Figure 3. Representative TLC fingerprints of eight species of the *Rhododendri* genus.

Species Comparison by HPLC

Figure 4 is the compiled HPLC chromatograms of all eight species. It is obvious that the fingerprint of each species was unique. By observing the HPLC fingerprint chromatograms of the rhododendri samples, only the *Rhododendron dauricum* L. contained six common peaks, including hyperoside, quercetin and farrerol.

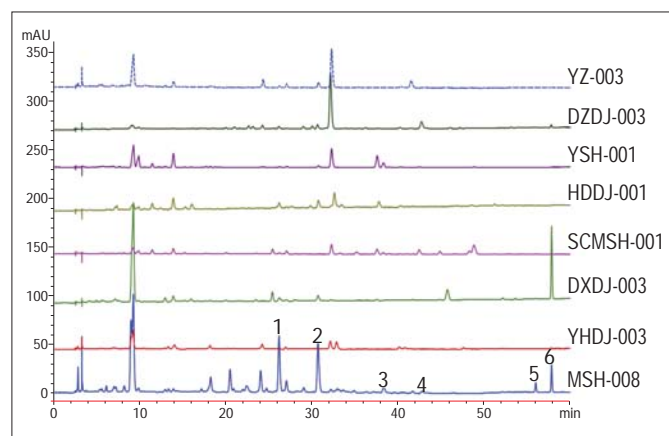


Figure 4. HPLC fingerprint chromatograms for eight species of the *Rhododendri* genus. (1) Hyperoside (M*), (4) quercetin, (6) farrerol (M*), and unidentified peaks (2), (3), (5). *M means marker

Principle Chemical Analysis

The HPLC data of the eight species of *Rhododendron* were imported into the PCA plot in order to authenticate *Rhododendron*. As shown in **Figure 5**, all ten batches of the *Rhododendri* Daurici Folium were significantly different from the rest. These principle chemical components were all belonged to a particular cluster and didn't overlap with each other. The result suggests that our HPLC method is useful and efficient for identification of MSH from other confusing species of the *Rhododendron*.

CONCLUSION

In traditional Chinese medicine, manshanhong has been used for anti-coughing and for eliminating phlegm. Pharmacological studies reveal that it has anti-tussive and expectorant effects and can inhibit allergic respiratory inflammation. However, because of many similar species either look alike or labeled with the same name, it is difficult to authenticate it from the others by organo-approach. The results of both TLC and

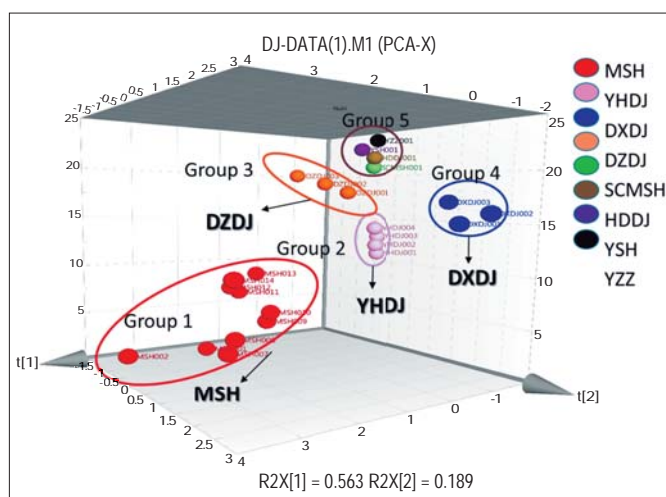


Figure 5. 3D Score cluster plot of various species of the *Rhododendri* genus based on six principal components (PCs). Red= MSH, pink= YHDJ, blue= DXDJ, orange= DZDJ, green= SCMSH, brown= HDDH, purple= YSH, black= YZZ

HPLC profile analysis in this study reveal that *Rhododendron dauricum* L. (Ericaceae) is different from other species of the *Rhododendri* genus. The present approach is a reliable and rapid method for its identification and quality control. Hence, our methods could be used for authentication and analysis of *Rhododendron dauricum* L. (Ericaceae).

ACKNOWLEDGEMENT

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Author's background

Ms HUANG Yeqing, Mr. ZHAO Hang, Mr. LEE Kin Ho, Miss LIN Yuxiu and Ms WANG Fang are research supporting staffs in Dr. Cheung's laboratory. They hold either a BSc degree or associate degree in Sciences. Dr. ZHANG Lehua is a botanist; he is an expert in breeding and cultivating plants. Dr. CHEUNG Hon-Yeung, who is a retire Associate Professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong, is currently a research fellow in the Department of Biomedical Sciences of the same university. He is a leader of the Research Group for the Bioactive Products in CityU. He is a manufacturing pharmacist and biotechnologist and has more than forty years of working experiences in industry, academic and consultancy jobs. He has been invited as an expert witness in court and a member of Biotechnology Committee for the Hong Kong and Shenzhen Government. Dr. Cheung has published more than 450 papers and articles in many prestigious international journals. His email address is cheung.honyeung@cityu.edu.hk

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Design and Production of a Chimeric VEGF Dimer for Regulatory Control of Metabolic Symbiosis and Micro-environmental Immunomodulation of Tumor Cells

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ABSTRACT

A recombinant gene coding for the vascular endothelial growth factor (VEGF) protein has been successfully constructed. This gene after proper transfect of a mammalian cell line expressed a chimeric fusion protein, VEGF₁₂₁-VEGF₁₆₅, which is a biologically active and functional protein capable to regulate cell proliferation, migration, invasion and tube formation of cancer cells as revealed in some *in vitro* and *in vivo* studies. Thus, this recombinant protein, may have great application as an anti-cancer drug to block angiogenesis and attenuate drug resistance of tumor cells via acting locally on the autocrine/ paracrine loop.

Keywords: Chimeric fusion protein, VEGF, Growth Factors, Anti-angiogenesis, Immunomodulation, Metabolic Symbiosis, cancer, PI3K-AKT-mTOR pathway

INTRODUCTION

Vascular endothelial growth factor (VEGF), originally known as vascular permeability factor (VPF),⁽¹⁾ is a signal protein expressed by cells that stimulates the formation of blood vessels. It is a sub-family of growth factors; the platelet-derived growth factor family of cystine-knot growth factors. VEGFs are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions.⁽²⁾ Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus.⁽³⁾ VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are

able to grow and metastasize. Thus, overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body.⁽⁴⁾ Drugs such as aflibercept, bevacizumab, ranibizumab, and pegaptanib can inhibit VEGF and could be used to control or slow those diseases.

Hence, angiogenesis is a critical rate-limiting process during tumor progression, which is induced by tilting the balance toward proangiogenic factors to drive vascular growth.⁽¹⁾ Cancer cells in a microenvironment count on angiogenesis to supply their need for oxygen and nutrients; thus, agents targeting angiogenesis pathway have been investigated as potential cancer drugs. However, VEGF-A targeting has not proven as efficacious as hoped because not all patients benefit from anti-angiogenic therapies.⁽⁵⁻⁸⁾ Most of the antiangiogenic agents achieve their effects by targeting VEGF-A and its VEGFR signaling, a cytokine that promotes blood vessel growth and tumor survival. The major question remained unresolved is to inhibit efficiently the activity of VEGF-A/ VEGFR signaling and alleviate hypoxia problem that drives resistance to antiangiogenic therapy.⁽⁹⁻¹²⁾ Therefore, we were wondering that whether we exploited the different binding efficiency of VEGF₁₂₁ and VEGF₁₆₅ to heparin, as a dimer, to compete efficiently the activity of VEGF₁₆₅ homodimer that triggers VEGFR signaling and to attenuate the activation of hypoxia signaling. In this study, we created and identified a novel chimeric dimer of VEGF₁₂₁ and VEGF₁₆₅, which was connected by Fc regions of human IgG1 to enhance dimerization. The chimeric VEGF₁₂₁-VEGF₁₆₅ has been examined to arrest the tube formation of endothelial cells and to interfere with growth, migration and invasion of cancer cells. We also found that the chimeric VEGF₁₂₁-VEGF₁₆₅ significantly inhibits angiogenesis *in vitro* of endothelial cells and cell migration/invasion of cancer cells in a paracrine and an autocrine manner. Furthermore, the fusion protein attenuated VEGFR2-HIF-1 α -VEGF₁₆₅/Lon signaling through PI3K-AKT-mTOR pathway in cancer cells. Our data demonstrated that the chimeric VEGF₁₂₁-VEGF₁₆₅ protein could be a potential drug as an antagonist to angiogenesis and to PI3K-AKT-mTOR and HIF-1 α signaling for future anti-angiogenic cancer therapy (**Figure 1**).

HYPOTHESIS AND EXPERIMENTAL APPROACH

As shown in **Figure 1**, Mechanism of VEGF₁₂₁-VEGF₁₆₅ targeting angiogenesis and alleviating hypoxia response in tumor metastasis and drug resistance. The diagram depicts the strategy of the actions of VEGF₁₂₁-VEGF₁₆₅ on endothelial cells and tumor cells to solve angiogenesis and drug restriction problems. The VEGF₁₂₁-VEGF₁₆₅ chimeric proteins competed endogenous VEGF₁₆₅ with VEGFR-2 receptor because the dimer VEGF₁₂₁-VEGF₁₆₅ possessed a higher mobility or affinity than endogenous monomer / dimeric VEGF₁₆₅ resulting in occupation of VEGF₁₂₁-VEGF₁₆₅ on receptor.

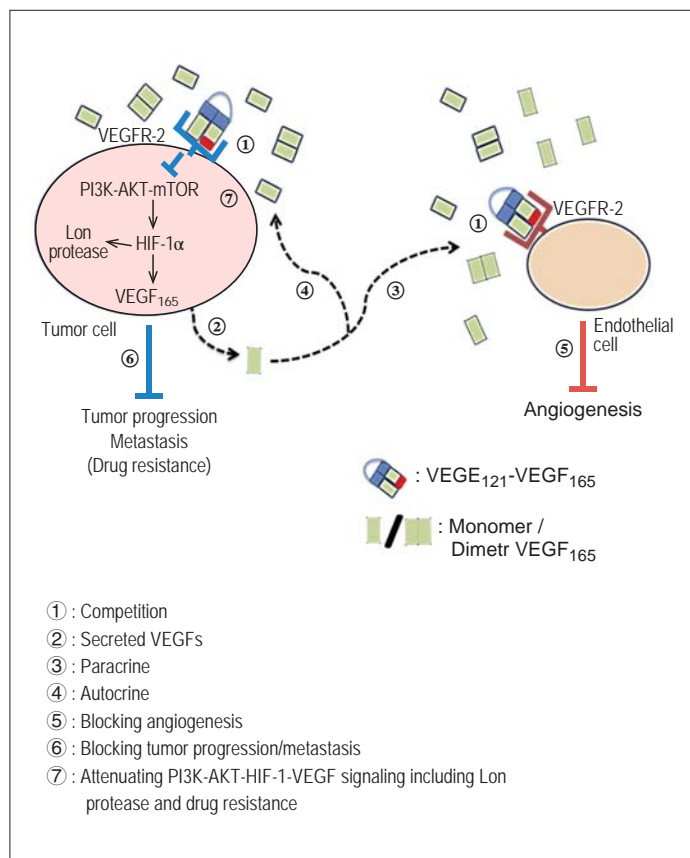


Figure 1. Research hypothesis and experimental design.

MATERIALS AND METHODS

Gene Construction of pcDNA3.1-VEGF₁₂₁-VEGF₁₆₅

The gene sequences comprised ORF of the human VEGF₁₂₁, VEGF₁₆₅, and IgG Fc, where the sequence encoding the linker is positioned between the sequences encoding the VEGF₁₂₁ and VEGF₁₆₅ (**Figure 2**). The VEGF₁₂₁-VEGF₁₆₅ gene fragment was inserted into a pcDNA3.1 plasmid. The integrity of final construct was confirmed by double digestion and DNA sequencing.

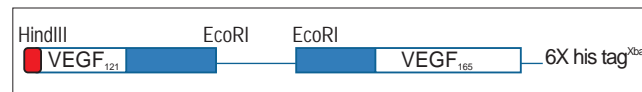


Figure 2. Design and construction of a VEGF Dimer Gene. The red box represents the gene coding for a signal peptide.

Expression of the Recombinant VEGF₁₂₁-VEGF₁₆₅ Protein in Human 293T Cells

The recombinant plasmids were transfected into 293T cells using the Biomics transfection reagent as described in the instruction manual of the pcDNA3.1 vector helper-free system (Biomics). After incubation for 36 h, the supernatants of the 293T cells were collected and purified on Ni-NTA resin, elute with 250 mmol/L imidazole according to the instruction manual. Finally, the purified proteins were confirmed by 10 % SDS-PAGE and Western Blot (**Figure 3**).

In Vitro Bioassay of Cell Proliferation, Cell Migration, Cell Invasion and Tube Formation

The proliferation of 3B-11 cells and HCT-8 cells were assessed using CCK-8 dye reduction assay (Enzo, USA), 3B-11 or HCT-8 were plated in 96-well plates and pre-treated with different concentrations of VEGF₁₂₁-VEGF₁₆₅ for 30 min and added with commercial VEGF₁₆₅ (Abcam, Cambridge, MA, USA) then incubated for 24h and measured it by ELISA (**Figure 4**). Corning Matrigel® Matrix (BD Biosciences, San

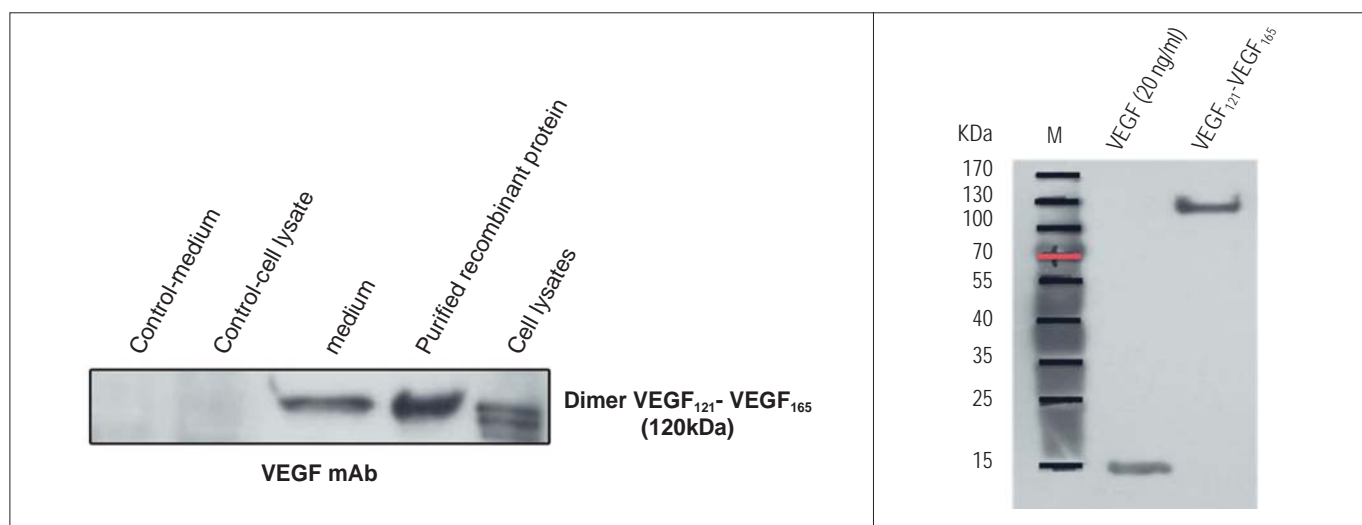


Figure 3. Western blot analysis of human recombinant protein VEGF₁₂₁-VEGF₁₆₅. The dimer recombinant protein was purified and elute by Ni-NTA column from medium. The arrow head indicated the target product, the MW was around 120 kDa. Western blot showed VEGF₁₂₁-VEGF₁₆₅ recombinant protein recognized by VEGF-A antibodies.

Jose, CA, USA) solution was thaw on ice overnight and 50 μ l aliquots were coated onto a 96-well plate and incubated at 37°C for 1h to solidify. 3B-11 cells were seeded onto the plated Matrigel Matrix and incubated at 37°C. Images of the formation of capillary-like structures were obtained after 2h with a computer-assisted microscope (Olympus, Tokyo, Japan) at 200x magnification (**Figure 5**). Cell migration assay was determined by gap closure assay. 3B-11 cells or HCT-8 cells were treated with different concentrations of the recombinant proteins for 16h. Cell migration can be monitored for 48h by microscope (**Figure 6**). Cells invasion were evaluated using a transwell chamber (Corning Costar; Cambridge, MA, USA) equipped with a Matrigel-coated filter membrane (8 μ m pores). Briefly, the filters were pre-coated with basement membrane proteins (Matrigel; BD Biosciences, San Jose, CA, USA) and allowed to dry overnight at 37°C with 5% CO₂. HCT-8 cells in FBS-free medium were seeded in the upper chambers, and lower wells were placed with 10% FBS medium. After incubation at 37°C for 48h, non-migratory cells on the upper side of the insert were removed with a cotton swab. The cells that had passed through the filter were fixed in methanol, stained with crystal violet. Randomly selected fields on the lower side of the photographed under microscopy were counted (**Figure 7**).

Investigation of Signaling Pathway of VEGF₁₂₁-VEGF₁₆₅ by Western Blot.

HCT-8 cells were seeded into 10 mm dish for 24h under normoxia and hypoxia (CoCl₂, Cobalt dichloride; 150 μ M) and treated as method previously described. Total protein concentrations were determined using a BCA protein assay. Equal quantities of total protein were resolved using 10% SDS-PAGE and electroblotted onto polyvinylidene fluoride membranes. Membranes were blocked with 5% skimmed milk and probed overnight at 4°C with primary antibodies. Membranes were then probed with the appropriate HRP conjugated secondary antibodies (GeneTex, Hsinchu, Taiwan) and the immunoreactive bands were visualized using an enhanced chemiluminescence method (Bio-Rad, Hercules,

CA, USA). Antibodies used in this study were purchased or produced as indicated: Antibodies to human Lon was produced as described previously. phospho-PI3K (Tyr458/Tyr199, #4228), phospho-AKT (Ser473, #4060), and phospho-mTOR (Ser2448, #2971) antibodies were from Cell Signaling Technology (Beverly, MA, USA); HIF-1 α (#610958) was obtained from BD Biosciences (Franklin Lakes, NJ); phospho-VEGFR2 (Tyr1054/Tyr1059, ab5473), VEGF-A (ab69479) from Abcam (Cambridge, MA, USA); beta actin from GeneTex (GTX109639, Hsinchu, Taiwan) (**Figure 8**).

Statistical Analysis of Data

Parametric Student's t test was applied in this study to assess the significance of difference between conditions of interest. In general, a p-value of < 0.05 was considered as statistically significant (Student's t test, *p < 0.05, **p < 0.01, and ***p < 0.001).

RESULTS

Construction and Expression of VEGF₁₂₁-VEGF₁₆₅ Fusion Gene

A VEGF₁₂₁-VEGF₁₆₅ fusion gene was cloned into the pcDNA3.1 vector, yielding the expression vector for VEGF₁₂₁-VEGF₁₆₅. The VEGF₁₂₁-VEGF₁₆₅ fusion gene (2394 bp) consisted of 408 bp of VEGF₁₂₁, 495 bp of mature VEGF₁₆₅, 711 bp of IgG1 Fc and 6X his tag gene. The plasmid of VEGF₁₂₁-VEGF₁₆₅ fusion gene was transfected into 293T cell line. The integrity of the construct was confirmed by DNA sequencing. It expressed two VEGF proteins connected by a polypeptide linker. The deduced protein consisted of 798-aa residues including a putative 26-aa signal peptide (**Figure 2**). The expression and secretion of VEGF₁₂₁-VEGF₁₆₅ fusion protein were confirmed by Western blot. It was found that a single protein band of ~120 kDa representing the VEGF₁₂₁-VEGF₁₆₅ fusion protein product was observed in the samples from the culture medium and cell lysate of transfected 293T

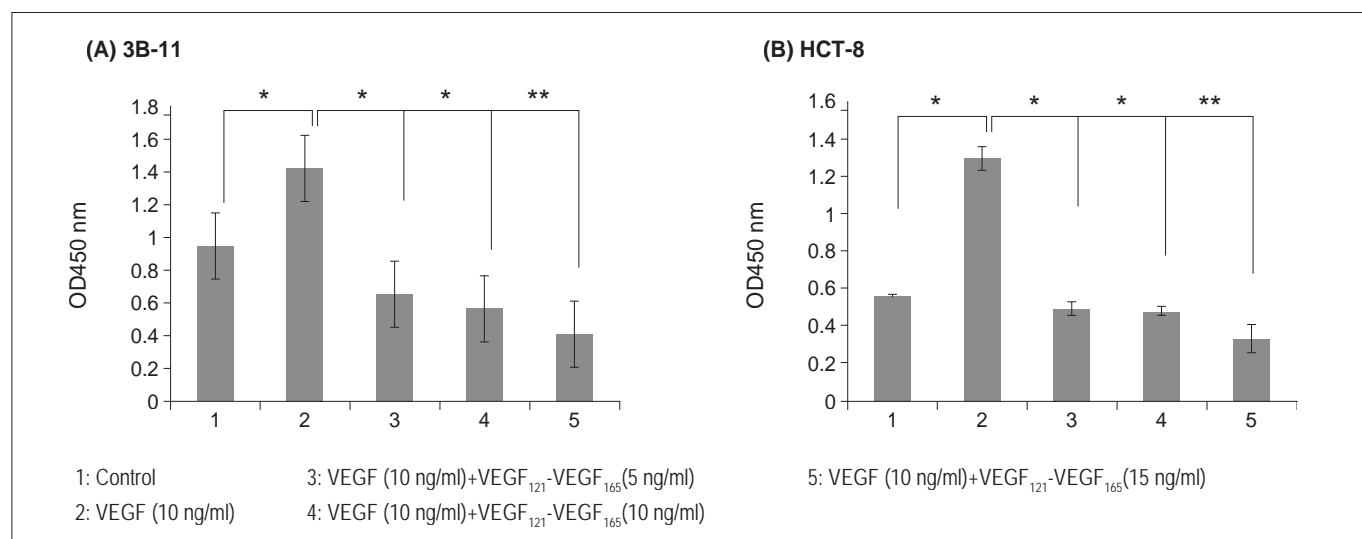


Figure 4. The 3B-11 and HCT-8 proliferation assay used to determine biology activity of recombinant protein VEGF₁₂₁-VEGF₁₆₅ expressed by 293T cells. The results showed 3B-11 HCT-8 were treated by VEGF₁₆₅ had higher values than seen in untreated control and experimental control.

cells. The recombinant protein VEGF₁₂₁-VEGF₁₆₅ was a His-tag fusion protein in the 293T cell. It was purified with the nickel affinity chromatography. Purity and molecular weight of the purified fusion proteins were determined by Western blot (Figure 3).

Effect of VEGF Dimer on Cell Proliferation

Since endothelial cell proliferation is required for the early angiogenic response, we examined whether the activity of

VEGF₁₂₁-VEGF₁₆₅ affected cell proliferation of VEGF₁₆₅ stimulated 3B-11 cell that is a convenient endothelial cell model for tube formation assay. VEGF₁₆₅-induced cell growth of 3B-11 was blocked in a concentration-dependent manner by VEGF₁₂₁-VEGF₁₆₅ (Figure 4). The effect of VEGF₁₆₅ using a concentration of 222 μ M on 3B-11 cell proliferation is easily inhibited by recombinant VEGF₁₂₁-VEGF₁₆₅ of 42 μ M, suggesting that VEGF₁₂₁-VEGF₁₆₅ is able to efficiently block the activity of VEGF₁₆₅-induced proliferation.

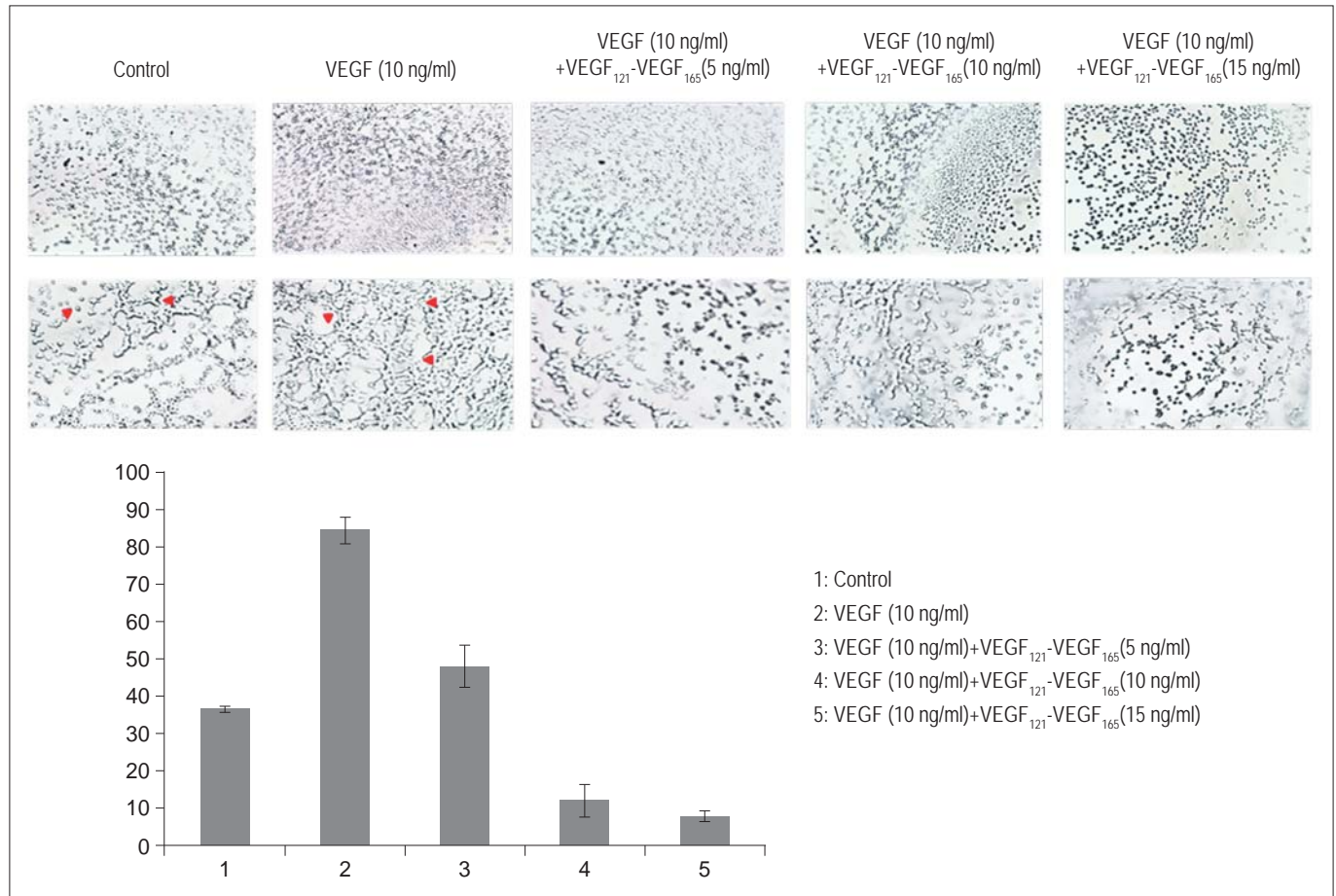


Figure 5. Effects of the VEGF₁₂₁-VEGF₁₆₅ on 3B-11 tube formation. 3B-11 (8×10^5 cells) were inoculated on the Matrigel and treated with VEGF (10 ng/mL), VEGF₁₂₁-VEGF₁₆₅ in the presence (5, 10, 15 ng/ml). Tube formation was quantified by counting the connected cells in randomly selected fields at 100x magnification.

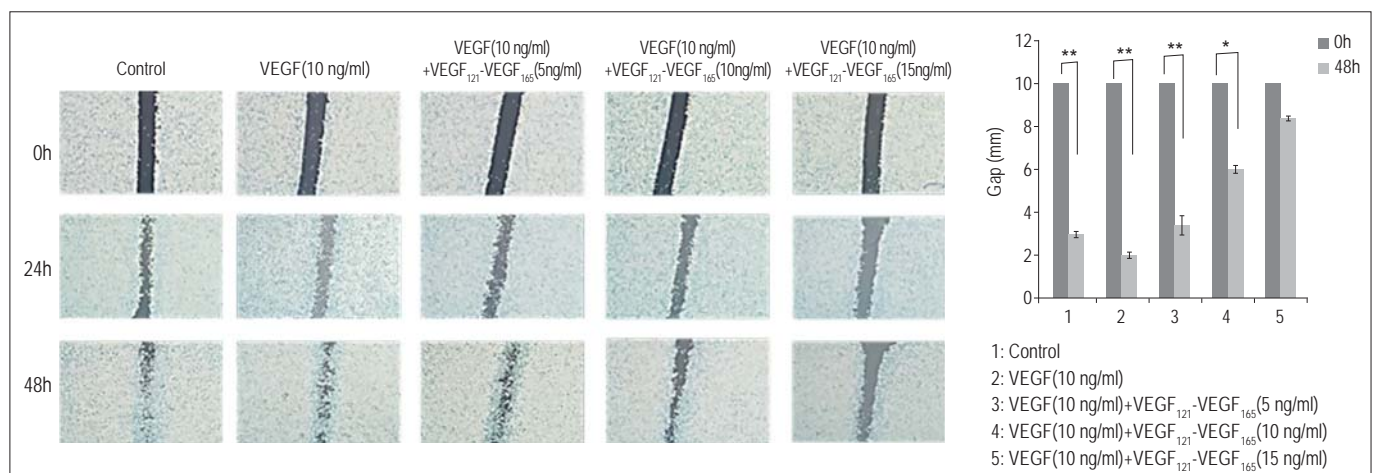


Figure 6. Migration abilities of HCT-8 cells. Migration of HCT-8 cells was enhanced in the presence of VEGF (10 ng/ml), recombinant protein VEGF (10 ng/ml) + VEGF₁₂₁-VEGF₁₆₅ (5, 10, 15 ng/ml) after 48 h as seen under a microscope. However, VEGF (10 ng/ml) + VEGF₁₂₁-VEGF₁₆₅ (15 ng/ml) was significantly inhibited cell migration.

Effect of VEGF Dimer on Tube Formation

It is commonly known that at later stages of angiogenesis it require morphological alterations of endothelial cells, which result in lumen formation. Hence, an *in vitro* tube formation assay in the presence of the chimeric protein was carried out by using 3B-11 endothelial cells that induce to invade a three-dimensional collagen gel where ever they form a network of capillary-like tubes. Our experimental data revealed that although 3B-11 cells form tube network under normal condition, i.e. in the presence of VEGF₁₆₅, numbers of tube formation is increased, the numbers of tube-like structure in 3B-11 cells dropped after the addition of VEGF₁₂₁-VEGF₁₆₅ in a concentration dependent manner (Figure 5).

Effect of VEGF Dimer on Cell Migration

Cell migration is a critical process in angiogenesis and tumor metastasis. The ability of cell migration was examined by a gap-closure migration assay. Consistently, the VEGF₁₂₁-VEGF₁₆₅ significantly inhibits migration of HCT-8. The results showed that the ability of cell migration in HCT-8 induced by VEGF₁₆₅ was inhibited by the addition of VEGF₁₂₁-VEGF₁₆₅ in a concentration-dependent manner (Figure 6).

Impairment of Tumor Invasion by Chimeric VEGF Fusion Protein

We also examined the effect of the chimeric protein on the activity of cell invasion by using the Transwell assay. Similar results were observed that, in the absence of VEGF₁₂₁-VEGF₁₆₅, VEGF₁₆₅ induced invasive capability as indicated by intensive penetration (Figure 7). However, the ability of VEGF₁₆₅-induced cell invasion was inhibited by the addition of VEGF₁₂₁-VEGF₁₆₅ in a concentration-dependent manner in HCT-8 cancer cells.

Effect of VEGF Dimer on Regulation of Angiogenesis-1 α -VEGF₁₆₅

We first examined the effect of VEGF₁₂₁-VEGF₁₆₅ on the VEGFR2-HIF-1 α -VEGF₁₆₅ axis in tumor cells. Since Lon is upregulated by the hypoxia inducible factor-1 α (HIF-1 α) and involved in a mechanism to response low oxygen availability and adapt cancer cells to a hypoxic environment,^(13,14) we also examined whether the fusion protein influences the expression of Lon protease. The level of all expression proteins was determined by Western blot analysis. The results show that the dimeric protein inhibited autocrine VEGFR2-HIF-1 α -VEGF₁₆₅/Lon signaling through PI3K-AKT-mTOR pathway in HCT-8 cancer cells (Figure 8).

DISCUSSION

Angiogenesis is a critical rate-limiting process during tumor growth, which is induced by tilting the balance toward proangiogenic status to drive vascular growth.⁽¹⁾ Many strategies have been designed to target the major proangiogenic VEGF-A/VEGFR signaling as cancer therapies, including small molecules inhibiting VEGFR signaling, anti-VEGF-A monoclonal antibody, VEGF-Trap, and anti-VEGFR antibody.⁽¹⁵⁻¹⁷⁾ One of several mechanisms of resistance to anti-angiogenic therapy is creating a hypoxic tumor microenvironment.^(18,19) In this study, we created and identified a novel chimeric dimer of VEGF₁₂₁-VEGF₁₆₅ fused with two Fc regions of human IgG1 as a powerful antiangiogenic modulator aforementioned *in vitro* and *in vivo* data. According to our results, the amount of VEGF₁₂₁-VEGF₁₆₅ chimeric protein used in the experiment to inhibit VEGF₁₆₅-induced activities ranged from 5 to 15 ng/mL, which is superior to RBDV-IgG1 Fc fusion protein. In addition, the amount of RBDV-IgG1 Fc fusion used in the suppression experiment on tumor growth *in vivo* is 150 μ g proteins of monomer.⁽¹⁰⁾ According to our preliminary results, VEGF₁₂₁-

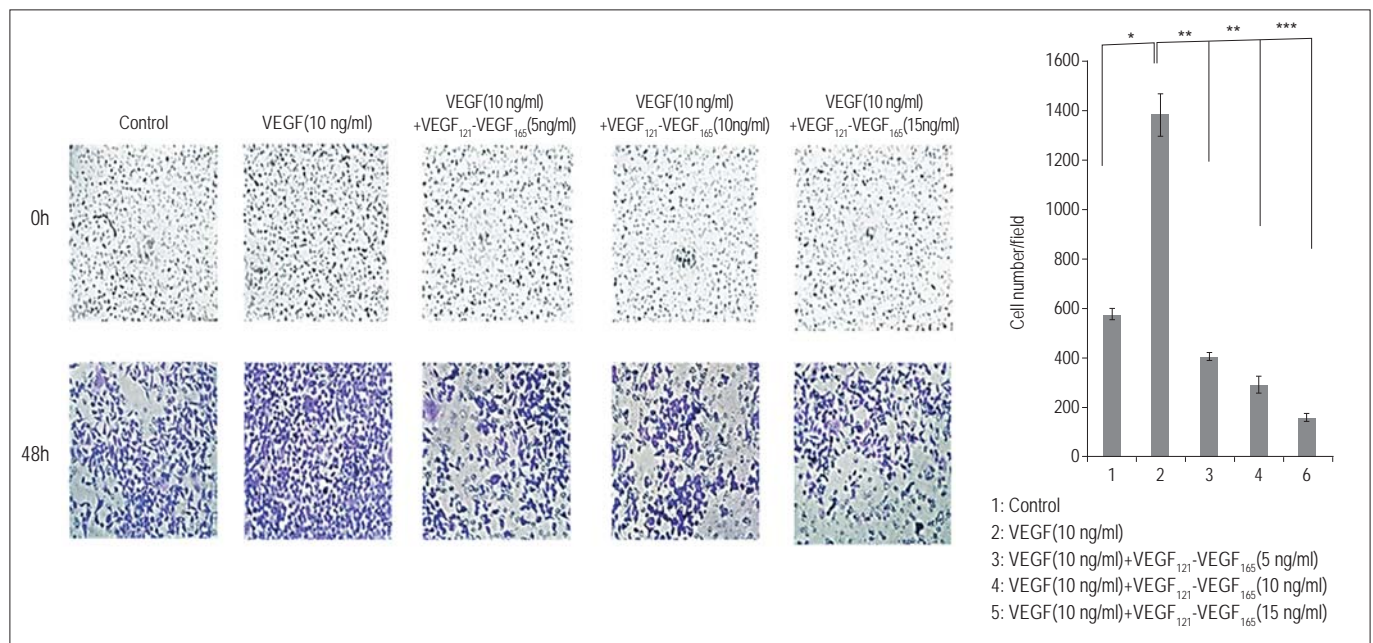


Figure 7. Inhibition of the chimeric dimer of VEGF₁₂₁-VEGF₁₆₅ recombinant protein *in vitro* cell invasion. HCT-8 cells were cultured with different concentration (5, 10, 15 ng/ml) of VEGF₁₂₁-VEGF₁₆₅ in the upper chamber of the transwell. After migrating for 48 h, cells on the bottom side of the filter were fixed, stained and counted.

VEGF₁₆₅ fusion heterodimer showed an *in vivo* inhibitory effect on tumor growth in mice after administration of the fusion protein ranging from 80 to 250 ng/ml (**Figure 9**). These findings suggest that VEGF₁₂₁-VEGF₁₆₅ chimeric fusion dimer has potential of great as an angiogenesis antagonist in future cancer therapy. (20) Our data demonstrated that the chimeric VEGF₁₂₁-VEGF₁₆₅ could be a potential drug as an antagonist to angiogenesis and PI3K-AKT-mTOR and HIF-1 α signaling in future cancer therapy, which will open up the patient opportunities to combat drug resistance to antiangiogenic therapy (**Table 1**). In the future, we will evaluate whether the VEGF₁₂₁-VEGF₁₆₅ improved both the hypoxia condition in tumor micro-acid environment (lactate) and immune cells to rectify the carbohydrate metabolic disorder via metabolic symbiosis and whether it also indirectly improved the expression of immunosuppressive factors as CTLA-4, T cells and NK cells are activated or not.

	Avastin	VEGF ₁₂₁ -VEGF ₁₆₅
Character	Monoclonal antibody	Fusion protein
Strategy	Neutralization endogenous VEGF ₁₆₅	Competitive endogenous VEGF ₁₆₅
Blocking Angiogenesis	Yes	Yes
Binding VEGFR2	No	Yes
VEGF(paracrine)	Inhibited	Inhibited
VEGF(autocrine)	Enhanced	Decreased
Hypoxia	Increased	Decreased
HIF-1 α	Increased	Decreased
Metastasis	Increased	Decreased
Dosage	<i>In vivo</i> : μ g	<i>In vitro</i> : ng <i>In vivo</i> : ng

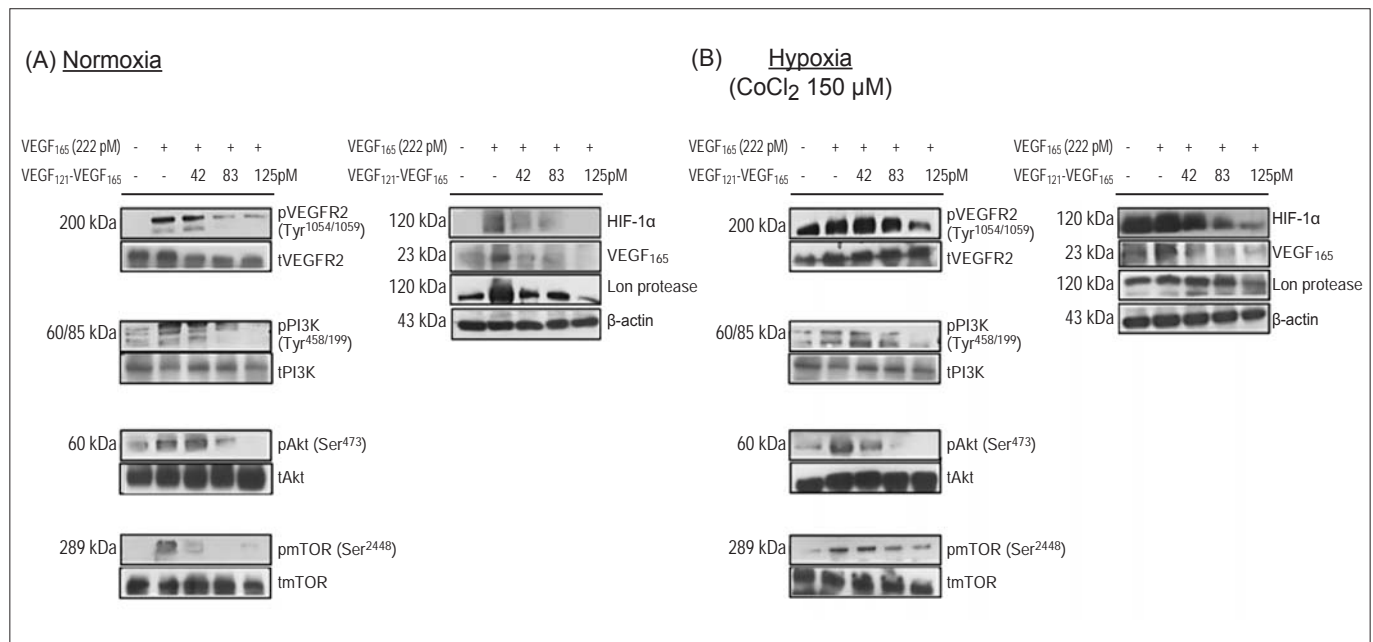


Figure 8. VEGF₁₂₁-VEGF₁₆₅ inhibited VEGF₁₆₅-induced activity through PI3K-AKT-mTOR-mediated VEGFR2-HIF-1 α -VEGF₁₆₅ / Lon pathway. HCT-8cells were treated with different concentrations of VEGF₁₂₁-VEGF₁₆₅ (42, 83, 125 pM) or not in the presence of VEGF₁₆₅ (222 pM) for 24 h under normoxia (A) and hypoxia (B). The expression level of the indicated protein was determined by using Western blot analysis.

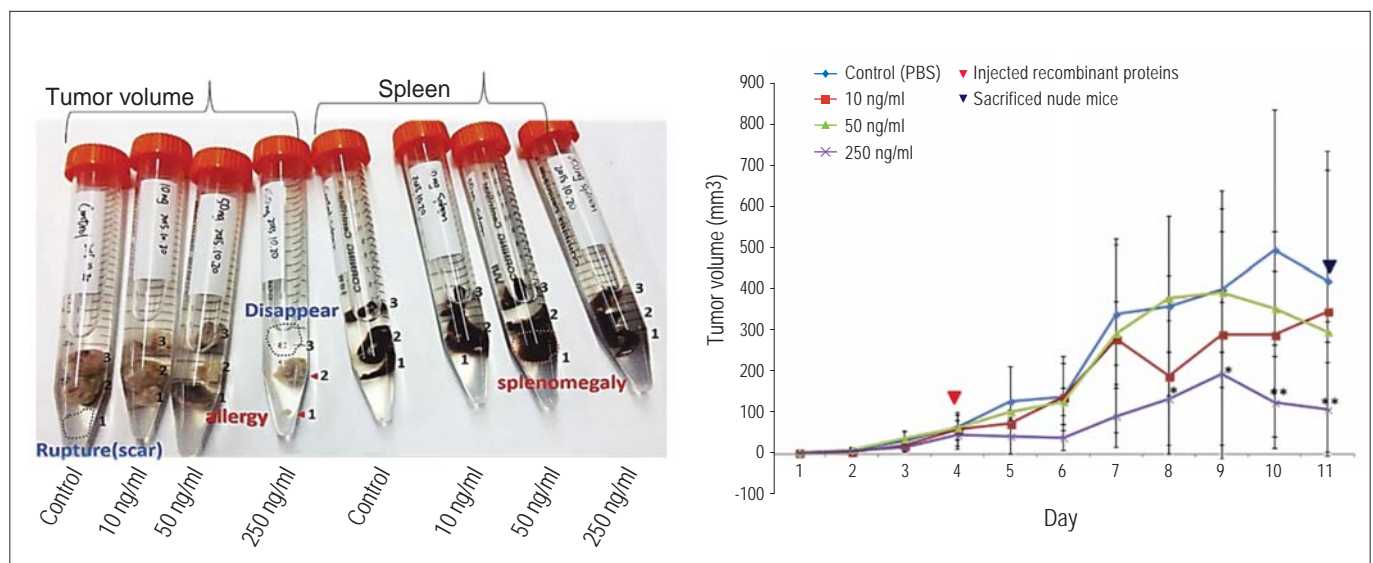


Figure 9. *In vivo* anti-tumor activity of VEGF dimer. The *in vivo* antitumor activity of VEGF₁₂₁-VEGF₁₆₅ fusion heterodimer was carried out in mice. The results show a positive result on suppression of tumor (Left diagram). Volume of tumor was monitored after *In vivo* administration of the fusion protein ranging from 4 to 12.5 ng/kg (Right diagram).

ACKNOWLEDGEMENTS

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Author's background

Dr. TSAI Jui-Ling, obtained her PhD in life science from National Taiwan University. She worked as a Post-Doctoral Fellow in Professor Newman Sze's laboratory at Nanyang Technological University in Singapore before joining Dr. Cheung's laboratory. Her main interest is in cancer research and drug discovery. **Dr. JIN Wei**, is a practicing medical doctor. She is currently working in an eye hospital in China. **Dr. CHEUNG Hon-Yeung**, who is an Associate Professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong, is a Manufacturing Pharmacist and Biotechnologist. He has more than 450 publications and received many awards for both of his research and academic works.

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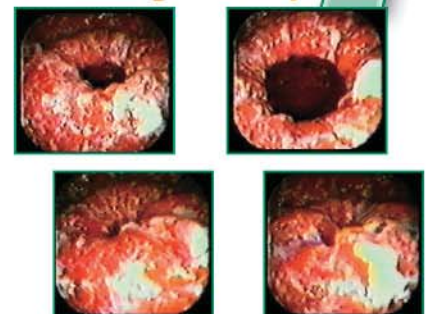
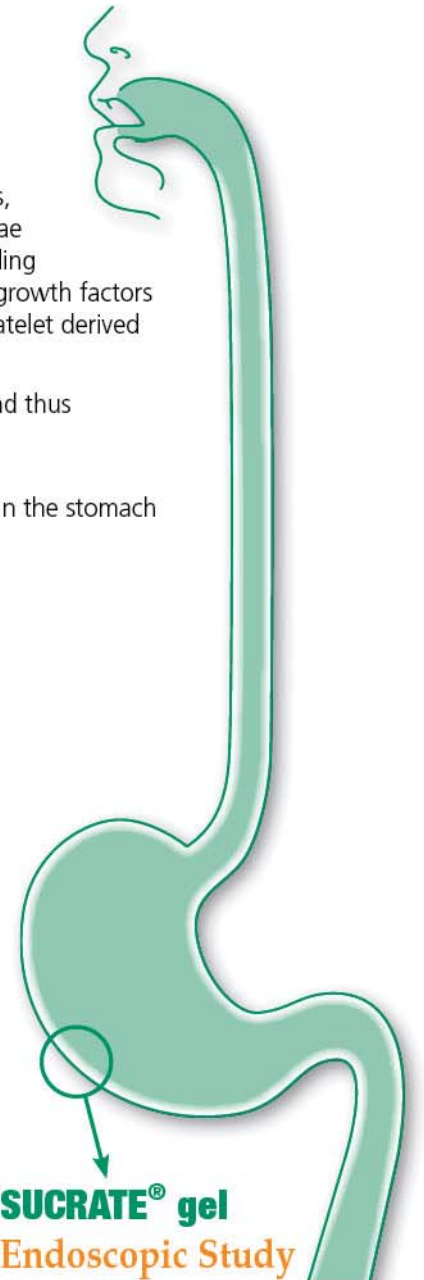
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Cosentino F. et al., Società Italiana di Endoscopia Digestiva, VII Simp. Naz, Napoli, 1992

Study on the Distribution of Crotonoside in Each Compartment of the Crotonis Fructus (Badou)

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ABSTRACT

Crotonis Fructus, which is a traditional Chinese medicine (TCM), has been used for a long time in China for water-expelling and purging cold stagnation of human body despite its high toxicity. A great deal of research efforts have been carried out on the analysis of the chemical components but none has been devoted to determination of bioactive components in each compartment of the fruit. Thus, there are some confusing or inconsistent statements amongst different regulatory compendia on its description and quality control. This study aims to provide some evidences so that an accurate scientific description could be proposed for the quality control of this TCM. Both methodologies of thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) have been developed and applied to this analytical study. Our results from both methodologies backed up each other that crotonoside, which is the most abundant bioactive component, was only found in the kernel but not in other compartment of the fruit. This suggests that the medicinal part of *Crotonis Fructus* is the kernel rather the whole fruit. Hence, it is clear that the kernel of *Croton* seed should only be used for enacting and optimizing the quality control of *Crotonis Fructus* in all pharmacopeias.

Keywords: *Crotonis Fructus*, Quality Control, Crotonoside, Magnoflorine, HPLC, HPTLC, Kernel, Regulatory Requirements

INTRODUCTION

In recent years, the toxicity of some TCM has drawn people's increasing attention. *Croton tiglium* L, which is one of the highly poisonous TCM, is widely distributed in tropical and subtropical zones, including India, New Guinea, Java, Indonesia, China and the Philippine islands yet it is commonly used by Chinese as a medicine.⁽¹⁾ The plant is shrubby and 12 m tall. Leaves are arranged in alternative, ovate to broadly rounded 4-9.5 cm. Flowers are generally small as shown in **Figure 1A & 1B**. Male flowers are generally star in shape with 15- 20 stamens. Hair and leaves are oblong ovate, Female flowers are apetalous. The capsule is triangular and scared with star shape hairs. It is 10-15 mm broad and 15-20 mm long.⁽²⁾ *Crotonis Fructus* is the fruit of *Croton tiglium* L. It is 1.8-2.2 cm long with diameter 1.4-2

cm long. It consists of three locules. Each locule contains one seed which is 1.2-1.5 cm long with diameter 0.7-0.9 cm long (**Figure 1C, 1D & 1E**).

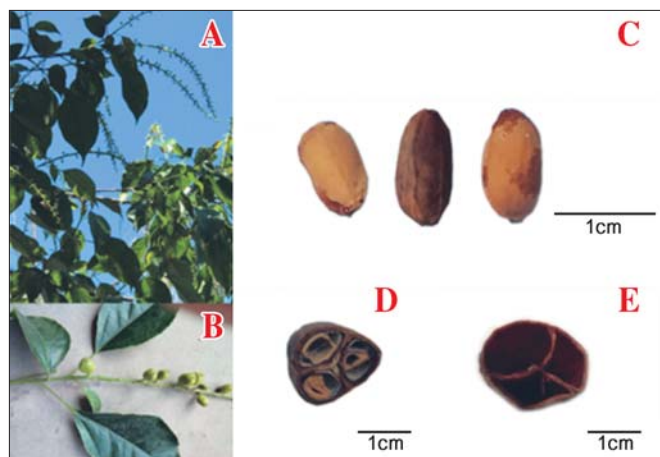


Figure 1. Morphological features of a plant and the fruit of *Croton tiglium* L. A: whole plant of *Croton tiglium* L; B: leaves and unripe fruits of the plant; C: magnified image of kernel; D: cross-section of the fruit showing seeds in 3 locules and E: empty locules

BIOACTIVE COMPOUNDS

Crotonis Fructus, which is commonly called "Badou" in China, has been used for a long time in China for water-expelling and purging cold stagnation despite its high toxicity.⁽³⁾ It is also reported to exhibit various biological activities like antimicrobial, proinflammation of gastrointestinal tract and anti-cancer.^(2,4,5) The major bioactive compounds are crotonoside (**Figure 2**), phorbol esters, crotonic acid and crotin.⁽⁶⁾ Crotonoside is also called isoguanosine. It is one of the natural-occurring guanosines and incorporated only in mammalian nucleic acids. It is reported to inhibit the growth of leukemia cells⁽⁷⁾ and treating gastrointestinal disorder through relaxing the intestinal smooth muscle.⁽⁸⁾ Phorbol esters are the ester derivatives of phorbol which is the hydrolysed product of toxic croton oil. It is reported to elicit inflammatory response through inducing expression of cyclooxygenase gene.^(4,5) Crotonic acid is another hydrolysed product of croton oil. It is revealed to inhibit bacterial growth through blocking the *gloeobacter* ligand-gated ion channel.⁽⁹⁾ Crotin, which is the major toxin of *Croton tiglium* L, is collectively

known as the toxic croton proteins.⁽¹⁰⁾ It is revealed to elicit proinflammatory response of gastrointestinal tract through activating mitogen-activated protein kinase signaling pathway.⁽¹⁾ Among those bioactive compounds, crotonoside is chosen as the chemical marker for authentication owing to its and specificity to *Croton tiglium* L and its high chemical stability, while others are not. For example, phorbol esters are abundant in species *Croton megalobotrys* Müll Arg. and *Jatropha curcas* L.⁽¹¹⁾ and crotonic acid are detected in seeds of *Daucus Carota* L.⁽¹²⁾ Although crotonin is present in *Croton tiglium* L. only, it is denatured easily upon heating due to its protein in nature.

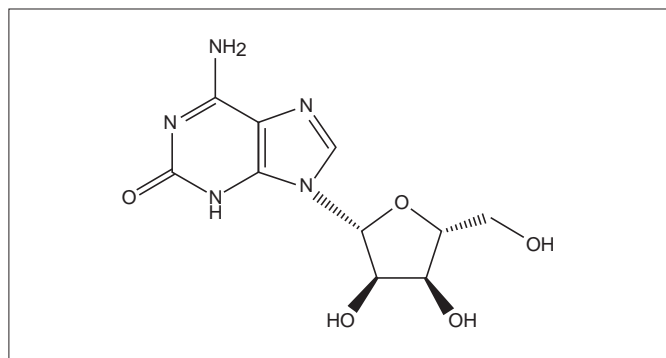


Figure 2. Chemical structure of crotonoside (syn. name: Isoquanosine)

ORIGIN OF THE PROBLEM IN QUALITY CONTROL OF CROTONIS FRUCTUS

Although a great deal of research efforts has been carried out on the analysis of its chemical components, none has been devoted to determination of bioactive components in each compartment of the fruit. Thus, there is discrepancy in statements amongst different regulatory compendia (Table 1).⁽¹³⁻¹⁶⁾ Such discrepancy imposes much difficulty on maintaining the TCM quality and identification of medicinal part. In this project we attempted to provide some data so that a more precise quality control plan of this TCM could be established. We applied different analytical methods to identify the location of the key pharmacological component in the fructus.

Pharmacopoeia or Ordinance	Name of CMM	Specifications
HK Cap 549 ^[7]	Unprocessed Crotonis Fructus (巴豆(生))	Unprocessed ripe fruit or seed of <i>Croton tiglium</i> L.
CP 2015 ^[8]	Crotonis Fructus (巴豆果)	Dried ripe fruit of <i>Croton tiglium</i> L.
THP 2013 ^[9]	Crotonis Semen (巴豆籽)	Dried ripe seed of <i>Croton tiglium</i> L.
KP 2012 ^[10]	Croton Seed	Seed of <i>Croton tiglium</i> L. from which testa has been removed

METHODOLOGY

Collection of Samples from Field: Ten batches of Crotonis Fructus samples were collected from different places of the mainland China. They were authenticated by Dr. Zhang Zhifeng (Faculty of Chinese Medicine, Macau University of Science and Technology, Macau).

TLC Identification: TLC separation was performed by a HPTLC silica gel F₂₅₄ plate. The mobile phase was composed of a mixture of n-Butyl alcohol, methanol, water and 25% v/v ammonium hydroxide (4:4:1:0.5 v/v).

HPLC Fingerprinting and assay: HPLC separation was performed by a reverse phase column (Zorbax SB-Aq column, 5µm, 4.6×250mm). The mobile phase was composed of 0.1% v/v phosphoric acid (A) and acetonitrile (B) using a linear gradient program of 0-5% (B) in 0-15 min, 5-35% (B) in 15-30 min.

RESULTS AND DISCUSSION

Pretreatment and Use of the Crotonis Fructus samples

Three out of the ten authenticated batches of Crotonis Fructus samples were used in this study. These three batches of fruit are shown in Table 2. The fruits were processed as described above prior to be analysed by TLC and HPLC methods.

Sample Code	Origin
SBD-01	Nanchong, Sichuan
SBD-02	Yulin, Guangxi
SBD-03	Yibin, Sichuan

Authentication of Crotonis Fructus by TLC Studies

Macroscopic and microscopic identifications are inadequate for TCM authentication. Chemical analysis is required since it reflects the medical values of a TCM as well as to distinguish one species from other one. In the TLC analysis, three batches of pulverized Crotonis Fructus samples were analysed. The three samples gave consistent results (Figure 3); the clearer sample band observation was achieved when kernel was used for extraction.

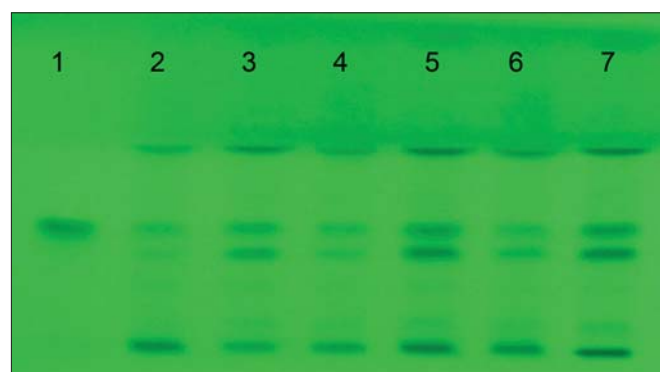


Figure 3. TLC sample lanes of Crotonis Fructus extracts. 1: crotonoside std stock, 2: extract of whole fruit of SBD-01; 3: extract of kernel of SBD-01; 4: extract of whole fruit of SBD-02; 5: extract of kernel of SBD-02; 6: extract of whole fruit of SBD-03; 7: extract of kernel of SBD-03.

Assay of Bioactive Components in Different part of Crotonis Fructus

Apart from rapid TLC analysis, HPLC analysis was performed since it reflected the true content of chemical components. According to the method developed our research team, a

typical chromatogram of kernel extract of *Crotonis Fructus* should give a fingerprint similar to **Figure 4**, in which the sample gives three characteristic peaks with acceptable retention times within the acceptable range of the corresponding peaks in the chromatogram. In the HPLC analysis, three batches of

pulverized *Crotonis Fructus* samples were analysed. Those three samples gave consistent results; Crotonoside was the most abundant bioactive compound in *Crotonis Fructus* (**Figure 5**). It was only found in the kernel (**Figure 5 & 6**). Although the chemical component profile of kernel was similar

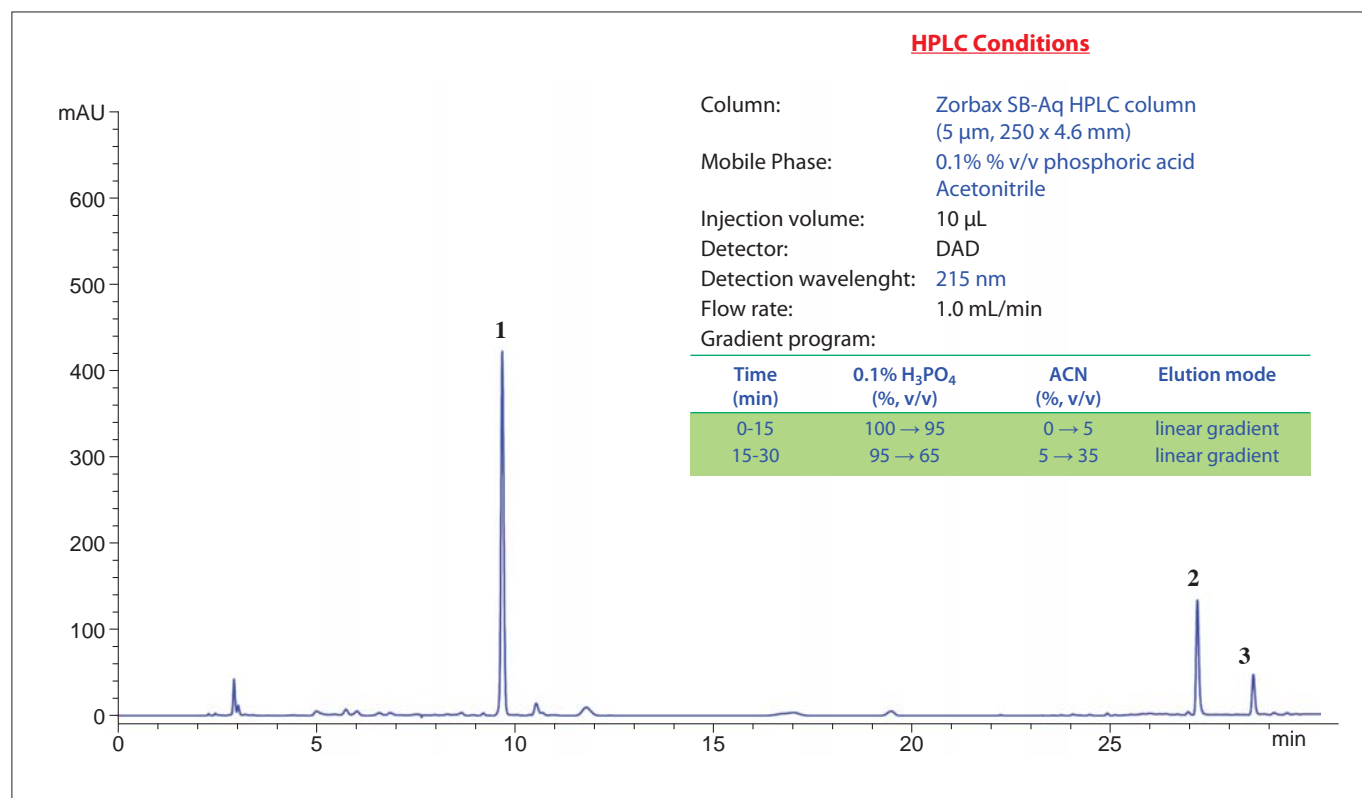


Figure 4. A reference fingerprint chromatogram of kernel extract of *Crotonis Fructus*. Peak 1: Crotonoside (RRT=1); Peak 2: Magnoflorine (RRT=2.80); Peak 3: Unknown compound (RRT=2.94).

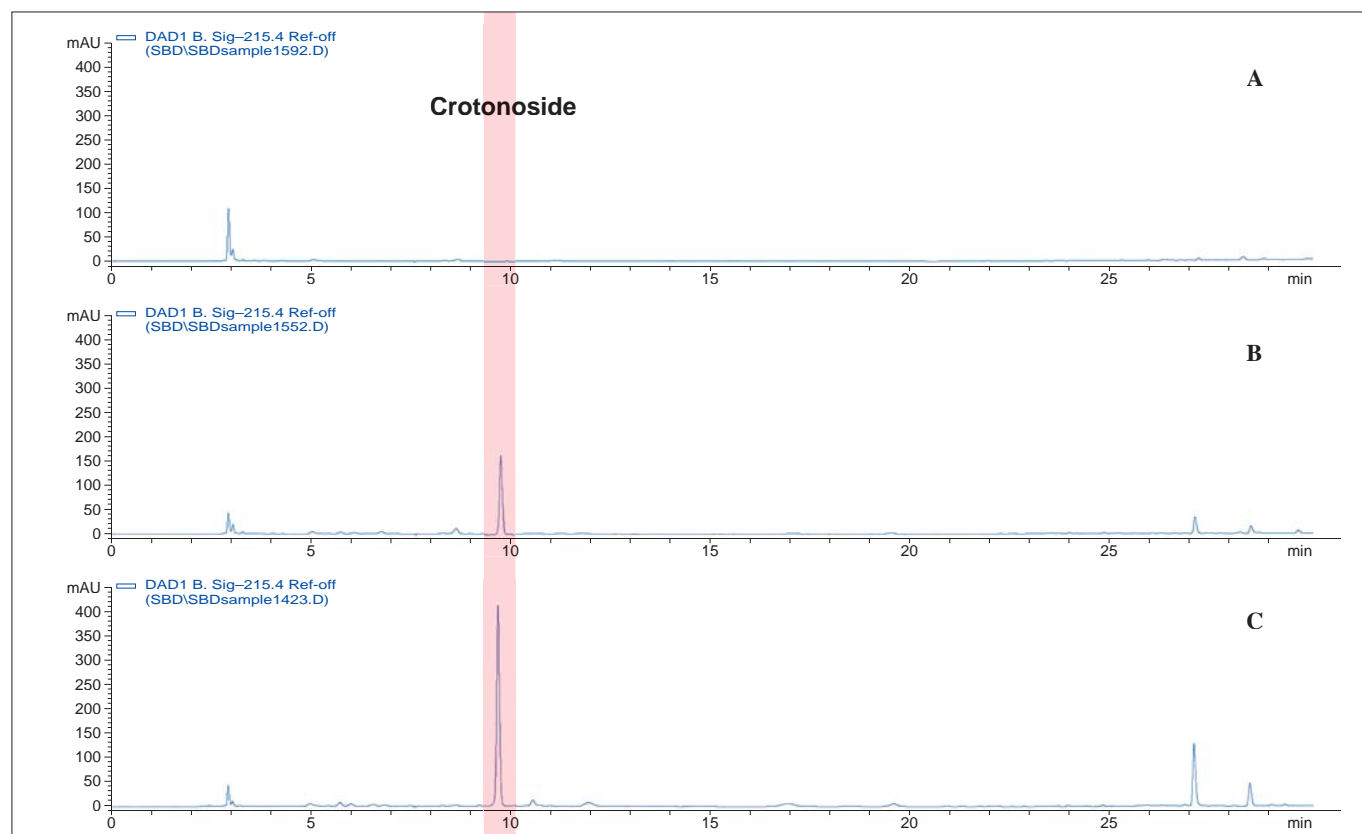


Figure 5. HPLC chromatograms of extracts of different compartments of unprocessed *Crotonis Fructus*. (A) fruit shell, (B) whole fruit, and (C) kernel of SBD-01

to that of whole fruit, crotonoside yield of kernel was about 2.5 times higher than that of whole fruit (Figure 6). The ratio shown in Table 3 supported that the lower crotonoside yield of whole fruit was attributed to the dilution of fruit shell.

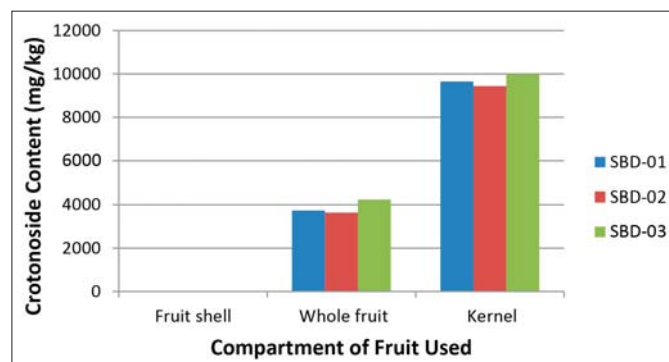


Figure 6. Content of Crotonoside in different compartments of Croton Fruit

Sample Code	Wt of Shell (g)	Wt of Kernel (g)	Shell / Kernel Ratio
SBD-01	32.71	71.01	1 / 2.171
SBD-02	28.58	75.74	1 / 2.650
SBD-03	29.46	76.31	1 / 2.590

CONCLUSION

The chemical component profile of the whole fruit is similar to that of the kernel but no crotonoside is detected in the fruit shell. The crotonoside yields of kernel are about 2.5 times higher than that of the whole fruit due to dilution of fruit shell. The results of TLC analysis are consistent with that of HPLC analysis. As a result, kernel instead of the whole fruit of the unprocessed Crotonis Fructus is advised for use in the authentication and medication.

ACKNOWLEDGEMENTS

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Author's background

Mr. LEE Kin Ho, Ms WANG Fang, Miss LIN Yuxiu and Mr. ZHAO Hang are research supporting staff. They have educational backgrounds in analytical chemistry. Dr. LAU, Terrence CK is associated professor in the Department of Biomedical Sciences of the City University of Hong Kong. Currently, he is the Associate Head of the department. He received both his undergraduate education and PhD degrees in bio-analytical chemistry from the Polytechnic University of Hong Kong. Before joining CityU in 2011, he worked as a research assistant professor in the School of Biomedical Sciences at the Chinese University of Hong Kong. Dr. CHEUNG Hon-yeung, who has retired in the summer in 2017, is currently working on contract term as a research fellow in City University of Hong Kong. He is a manufacturing pharmacist and biotechnologist. He has published more than 450 papers and received many awards for both his research and academic works. Before retirement, he was an associated professor of Pharmaceutical Microbiology & Biotechnology in CityU.

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Student's Experience on Attending the 2018 Forbidden City International Pharmacist Forum

LAU, Tsz Chun

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As we have stepped into an era of globalization, it is essential for pharmacy students building up a multifaceted understanding of pharmaceutical care. The 2018 Forbidden City International Pharmacist Forum is an excellent opportunity for me to explore the pharmaceutical services and role of pharmacists outside Hong Kong. This year, the forum is themed on "Patient's Medication Safety and Challenges - Pharmacists are in Action". Many seminars and discussions based on the practice of pharmacists are held. Apart from the forum, we also visited the Beijing United Family Hospital, which was the first foreign-invested hospital to operate in China. The hospital has imported equipment, modern facilities and well-trained professionals. The trip was a fruitful experience that we learned the ongoing trend of pharmaceutical care. The PSHK delegation was led by Ms. Mary Cheng, together with Mr. Benjamin Kwong, Ms. Teresa Ngan, Ms. Christina Leung, Prof. Cheung Yin Ting, and Mr. Lau Tsz Chun (**Photo 1**).

BEIJING UNITED FAMILY HOSPITAL

On the first day, we visited the Beijing United Family Hospital and Beijing United Family Rehabilitation Hospital (**Photo 2**). Founded in 1997, Beijing United Family Hospital (BJU) was the first international standard hospital established in China. BJU is accredited by both Joint Commission International (JCI) and the College of American Pathologists (CAP). It has also received Stage 6 EMRAM certification from the Healthcare Information and Management Systems Society (HIMSS). Moreover, there are over 200 full-time senior physicians and specialists from all over the world to provide professional expertise, for example, U.S., U.K. and Australia. The hospital is devoted to offering premium care to hundreds of thousands of expatriate and Chinese families.



Photo 1. Left to right (Back row: Prof. Andrew McLachlan, Ms. Christina Leung, Ms. Carolina Ung, Ms. Teresa Ngan; Front row: Mr. Lau Tsz Chun, Ms. Mary Cheng, Mr. Benjamin Kwong, Prof. Cheung Yin Ting)



Photo 2. Visit at Beijing United Family Hospital (BJU)

The pharmacy department has 24 pharmacists, 4 resident pharmacists, 3 technicians and 5 stock management staff. They are responsible for serving inpatient pharmacy, outpatient pharmacy and 8 clinics (satellite pharmacy) outside the hospital. They process 1939 prescriptions per month. For the inpatient pharmacy, electronic prescription system is utilized. Prescriptions are received from doctors and verified by pharmacists. The medications are then picked up

by dispensers, double-checked by pharmacists and finally sent to wards. As BJU is a small-scale private hospital, the medications for inpatient use can be stored in a small room. Dispensers can pick up the correct medications in a more efficient way. Currently, the inpatient pharmacy is planning to launch the unit-dose system in the coming year. To simulate the unit-dose system, the pharmacy arranged some of the drugs in a way that is as same as that in the machine. Pharmacists and dispensers can get themselves familiarized with the location of medication before the launch of the machine (Photo 3).

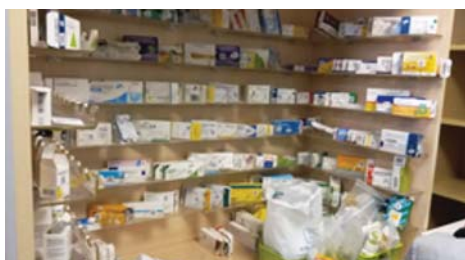


Photo 3. Simulation of unit-dose machine in inpatient pharmacy

We also visited the outpatient pharmacy. The stock covers different types of medications, including Traditional Chinese Medications, for example, Nin Jiom Pei Pa Koa Syrup 念慈菴川貝枇杷膏, Xiao Yao Wan 逍遙丸 and Ren Shen Gui Pi Wan 人參歸脾丸 (Photo 4). Every product in pharmacy is stuck with a QR code label, which can provide information including name of product, indication, batch number, expiry date, etc. These information can be accessed by pharmacy staff as well as patients. As the patients in BJU are usually well-educated, they may request more medicinal information. Therefore, the QR code tool can satisfy their demands.



Photo 4. TCMs in outpatient pharmacy

BEIJING UNITED FAMILY REHABILITATION HOSPITAL

Beijing United Family Rehabilitation Hospital is situated at another corner of the city. It practices evidence-based medicine in staged assessment of patients and rehabilitation planning. Individualized rehabilitation programs based on patients' needs are also provided. The professional team consisting of TCM doctors, psychologists, clinical pharmacists, physiotherapists, registered dietitian and case managers, is highly proficient, experienced and multinational. The services and

programs cover neurologic rehabilitation, orthopedic and sports rehabilitation, cancer rehabilitation, women's health, pediatric rehabilitation and palliative care. The hospital also adopts advanced therapy and equipment, physical therapy, hyperbaric oxygen therapy, aquatic rehabilitation (Photo 5), to name a few. The living environment is comfortable and quite, so patients can be relaxed in their rehabilitation journey. The hospital provides high-quality service that minimizes the consequences of diseases, restores self-confidence and quality of life.



Photo 5. Aquatic rehabilitation

2018 FORBIDDEN CITY INTERNATIONAL PHARMACIST FORUM

The 2018 Forbidden City International Pharmacists Forum was held in Beijing Conference Center from May 11 to May 13. "Patient's Medication Safety and Challenges - Pharmacists are in Action" was selected as the 10th anniversary theme. (Photo 6). Topics including the establishment of drug injury prevention mechanisms, medication errors in hospital and system management, pharmacist values in health management, chronic disease management and prevention of medication injury, children's drug development and clinical applications, and also the elderly patients care are discussed. The forum has invited top-level experts from all over the world to share their views on pharmaceutical care.



Photo 6. Theme of 2018 Forbidden City International Pharmacists Forum

The theme of the opening keynote speech is the prevention of drug hazard and establishment of its mechanism. Ms Mary Cheng was the moderator of 3 sessions followed by the opening ceremony (Photo 7). Dr. Paul W. Abramowitz,



Photo 7. Ms Mary Cheng (left) as the moderator of 3 sessions in the opening ceremony

the Chief Executive Officer for the American Society of Health-System Pharmacists (ASHP), gave us a talk on “Transforming Patient Care: Pharmacy Practice Model Change”. The presentation discusses the practice model change, new methods of care and advance pharmacotherapy in ambulatory settings. The pharmacy practice model also includes leveraging pharmacy technicians, pharmacist credentialing and training, technology, and pharmacy leadership. It is a very inspiring talk which deepens my understanding on advanced pharmacy practice in ambulatory care settings.

Ms Teresa Ngan, Mr Benjamin Kwong and Prof. Cheung Yin Ting were invited to give their presentations in the forum. Their sharing covered different areas of pharmaceutical care and inspired us of new thoughts.

Ms Teresa Ngan was invited as a speaker in the seminar “Pharmacy Automation and Medication Safety” (**Photo 8**). She focused on the modernization of pharmaceutical services through advanced technology. The roadmap in Strategic Plan for 2017-22 in Hospital Authority was introduced. To meet the increasing demand for pharmaceutical services, HA has strengthened the use of information technology to facilitate computerized decision support and enhance medication safety through closed-loop medication management. The introduction of IPMOE has enhanced pharmaceutical care, empowered healthcare professionals in accessibility to drug information, efficiency and optimal use of drugs. The presentation well depicted the use of technology in HK hospitals and received positive feedback from other speakers.



Photo 8. Ms Teresa’s sharing on modernization of pharmaceutical services through advanced technology

Mr Benjamin Kwong was responsible for the last speech in the seminar “Pharmaceutical Care in Hong Kong, Macau, Taiwan and mainland China” (**Photo 9**). He stressed the importance of raising the standard of pharmaceutical services. Facing the growing drug development and generalization of personalized medication, pharmacists ought to do continuous education and keep updated on therapeutics knowledge. Pharmacists should consider a basket of factors when treating patients, for example, healthcare cost, patient compliance, expected benefits and drawbacks, etc. As the last speaker

of the seminar, he well concluded the role of pharmacists, how pharmacists could improve their quality and extend their role in patient care. A brisk discussion also followed after his presentation. Participants actively exchanged ideas and deepened understanding of the topic.



Photo 9. Mr Benjamin Kwong’s sharing on “Energise Pharmaceutical Care Services - Start by Pharmacists”

Prof. Cheung Yin Ting gave her sharing on her research in the seminar “Medication and Risk Management of Women and Children”. Her topic was “Improving Clinical Care in the Pediatric Population through Pharmacy Practice Research” (**Photo 10**). She highlighted the challenge in the use of drugs in pediatric population due to lack of evidence in dosing strategies and inadequate knowledge of the adverse impacts on the child’s long-term health outcomes. The discussion is centered on pediatric oncology, as well as critically ill patients in the PICU/NICU. It aims at inspiring collaborative research within the region to advance clinical care in children. Her sharing received positive feedback from the academic executive chairmen, who praised that her research would be a contribution to pediatric care.



Photo 10. Prof. Cheung Yin Ting’s sharing on “Improving Clinical Care in the Pediatric Population through Pharmacy Practice Research”

I also attended other seminars and listened to presentations hosted by experts from all around the world, including Ms Carolina Ung from Macau, Prof. Andrew McLachlan from Australia and Prof. Toshiaki Sendo from Japan. They enlightened me on the idea of pharmaceutical care in

a global setting. Moreover, I also watched “The Final of the 4th Competition for Chinese Pharmacist Professional Skills” in which pharmacists from 12 hospitals in different regions of China engaged in a competition covering pharmacology, prescription verification, patient counselling and therapeutics knowledge (**Photo 11**). The competition was fierce and competitors were of high standard. The competition enriched our knowledge and encouraged continuous education. We Hong Kong may consider organizing a small-scale competition for pharmacists.



Photo 11. The Final of the 4th Competition for Chinese Pharmacist Professional Skills

PERSONAL REFLECTIONS

The forum provided me with insight for the role of pharmacists in different settings. The pharmaceutical service in Hong Kong still has room for improvement. Although the pharmacy industry is now facing challenges, there are also opportunities for us. We need to make good use of the growing labor in the market and expand the scope of pharmaceutical services. We should set a positive image of pharmacists and let the public know our roles in primary healthcare. The forum enlightens me on the potential development of pharmaceutical care. We can learn from experience of other countries and make every effort to improve our services.

Again, thank you for PSHK sponsorship. I am very delighted to join the forum and adsorb ideas from experts from all over the world. It is eye-opening that helps me understand pharmaceutical care in a global context. It also gives me insight into future career development. It is a very fruitful and stimulating professional development experience. I highly recommend all of you to join the forum.

Author's background

Mr. Lau Tsz Chun is a third year Pharmacy student of the School of Pharmacy, the Chinese University of Hong Kong. His corresponding email address: ansonlau97@gmail.com

SHPHK – New Term, New Vision

Reported by **Vienna Leung**, Pharmacist of the Society of Hospital Pharmacists of Hong Kong

The Annual General Meeting (AGM) of the Society of Hospital Pharmacists of Hong Kong (SHPHK) was successfully held on 6th April 2018 at the Cityview Hotel, Yau Ma Tei. This year, we are especially pleased to have members from different private hospitals to join the Committee of the Society. At the meeting, Mr. William Chui, President of SHPHK, once again emphasized the importance of the reinforcement of collaboration between public hospitals and the private healthcare sector through the Public-Private Partnership (PPP) Programme. SHPHK will continue to work with different parties to advance the pharmacy practice in Hong Kong.



Mr. Michael Ling, Honorary Advisor of SHPHK, presenting the progress report on patient educational activities organised by the Society in 2017 at the AGM.

The 'e-Drug Info' App (「知藥易」應用程式)

The SHPHK is proud to announce the launch of its new 'e-Drug Info' mobile App. The App was launched on 30th April following a press conference on 23rd April, and has attracted coverage by some media.



A press conference announcing the launch of the 'e-drug info' app was held by the Society on 23rd April 2018. From left: President Mr. William CHUI and General Committee member Ms. Vivian NGAI.

The 'e-Drug Info' App provides accessible and reliable drug information to the public, including information on indications, common side effects, medical assistance programmes, etc. It contains a series of educational videos on using different inhaler devices, as well as drug information for some of the latest cancer drugs. It also provides an innovative and convenient way for the public to find a drug by simply scanning the barcode on the outer package of the drug product.

For IOS device



For Android device



The App is now available on both IOS and android devices. Just scan the following QR code with your smartphone to download the App now!

At this stage, the App covers drug information and educational videos for most of the inhalers and some of the cancer drugs. The Society will continue to expand the drug database for the best benefits of the general public.

Road to Success – How to prepare for a job interview, a sharing session by Mr. Michael Ling



A sharing session on 'How to prepare for a job interview' was held on 26th May 2018 at the SHPHK Clubhouse.

Good interview skills may get you a job offer, but how can we be sure that we do not make silly mistakes at an interview? We were honored to invite Mr. Michael Ling, an inspiring leader and Honorary Advisor of SHPHK, to share with our student

and intern Members the essential skills for a successful interview. It was a relaxing and interactive afternoon session, which allowed attendees to raise questions concerning interview preparation. The Society would like to thank Michael for his precious time to share his invaluable experience with our pharmacists-to-be!

SHPHK Hiking Trip 2018

Besides educational activities to help its members to keep pace with the latest medical and pharmaceutical development, SHPHK also organizes a number of team-building activities, with an aim to foster communication



The spectacular view of Tung Lung Island!

between members and the Society. A hiking trip to Tung Lung Island was organized on 27th May 2018. Despite the extremely hot weather on the day, it was a fun, rewarding, and enjoyable experience for everyone who joined the trip!

If you would like to get involved in the activities organized by SHPHK, feel free to join us as member to enjoy the benefits of the Society, and work with us to promote the betterment of the pharmacy profession together!

You are most welcome to follow the Society's Facebook page (@SHPHK) to know more about the Society's development and activities. You may also visit the Drug Education Resources Centre (DERC) Website: www.derc.org.hk to keep abreast of the latest news and development of drugs in Hong Kong. Join us now as new member or renew your membership at the Society's website: www.shphk.org.hk.

The Society of Hospital Pharmacists of Hong Kong (SHPHK) Office Bearers 2018/2019

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Chief Editor:	CHU Man Wa Amy
Editors:	NG Man Keung
	YIU Sui Ki Kenneth

PSHK Upcoming Continuing Education Programmes for Pharmacists

Reported by **Scarlett PONG, BBS, JP, President**, The Pharmaceutical Society of Hong Kong

The Pharmaceutical Society of Hong Kong has been working on various new initiatives for promoting pharmacist roles to the public during the first half of 2018, including pharmacist medication management service programmes. We are glad to let you know more exciting programmes for our pharmacists will be upcoming!

Professional Training for Pharmacists on Dementia Care

A training course on dementia care specially for pharmacists would commence on the 22nd and 29th of July.

To align with the Government's policy direction on primary care and disease prevention, it is important for pharmacists to equip ourselves with more knowledge and skills, so as to extend our roles and actively engage in the primary care collaboration model. Primary and secondary prevention are important particularly for the non-communicable diseases (NCDs), including dementia, as they require long-term management and impose very heavy burden on our healthcare system. Therefore, PSHK liaised with Jockey Club Centre for Positive Ageing to offer a training course on dementia care specially for pharmacists.

The course will introduce the early detection of dementia and mild cognitive impairment (MCI), practical skills of cognitive assessments, interventions of dementia and community care for elderly with dementia. In fact, the assessment methods introduced in this training program including Mini-Cog and MoCA were not performed only by medical practitioners, but also by other health professionals including nurses, occupational therapists and physiotherapists etc. By acquiring such skills, pharmacists can also participate in dementia assessment services in the future. For example, PSHK has also been very actively providing pharmacist medication management service, drug education and disease prevention in Kwai Tsing

and in other districts. We will also engage pharmacists to NCD screening and dementia assessment services in the upcoming future.

Advancing Hong Kong Pharmacy Profession Development – Local Continuing Education Programmes

Another great news is that our Society has successfully got the funding from Professional Services Advancement Support Scheme (PASS) offered by The Commerce and Economic Development Bureau to launch a 3-year continuing education programme for our local pharmacists!

The programme aims to upgrade our local pharmacist's knowledge on chronic disease prevention and management, as well as on elderly services to align with our Government's healthcare development direction. The programme will consist of the following activities:

- (1) Twelve education seminars in Hong Kong on professional pharmaceutical knowledge
- (2) One international symposium in Hong Kong on latest advancement in pharmaceutical services with overseas speakers
- (3) One systematic survey for future establishment of pharmaceutical continuing education framework in Hong Kong

The programme shall start in September this year and lasts till 2021. The first two seminars will be about 'Common geriatric syndromes' and 'Cardiovascular disorders' to be held in the fourth quarter of 2018. All the seminars will be offered free to our local pharmacists! We recommend you to enrol in all or as many as possible of the seminars so to gain a Platinum certificate of attendance. So please stay tuned with our Facebook, website and mass mail for updates for these seminars!



Kwai Tsing District Primary Care Services Promotional Activity

NEW INDICATION

KEYTRUDA® (MSD)

Prepared by Lucilla Leung

Active Ingredient:

KEYTRUDA Solution for Injection 100mg/ 4ml: carton containing one 100 mg/4 mL (25 mg/mL), single-use vial.

KEYTRUDA for injection (lyophilized powder): carton containing one 50 mg single-use vial.

Indications:

1.1 Melanoma

KEYTRUDA (pembrolizumab) is indicated for the treatment of patients with unresectable or metastatic melanoma [see Clinical Studies (14.1)].

1.2 Non-Small Cell Lung Cancer

KEYTRUDA is indicated for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have high PD L1 expression [Tumor Proportion Score (TPS) $\geq 50\%$] as determined by a validated test, with no EGFR or ALK genomic tumor aberrations [see Clinical Studies (14.2)]. KEYTRUDA is indicated for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 (TPS $\geq 1\%$) as determined by a validated test with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on approved therapy for these aberrations prior to receiving KEYTRUDA [see Clinical Studies (14.2)].

1.3 Urothelial Carcinoma

KEYTRUDA is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy see Clinical Studies (14.3)].

KEYTRUDA is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy

Dosage and Administration:

2.1 Patient Selection

Select patients for treatment of metastatic NSCLC with KEYTRUDA based on the presence of positive PD-L1 expression [see Clinical Studies (14.2)].

2.2 Recommended Dosage for Melanoma

The recommended dose of KEYTRUDA is 2 mg/kg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity [see Clinical Studies (14.1)].

2.3 Recommended Dosage for NSCLC

KEYTRUDA should be administered as an intravenous infusion

over 30 minutes every 3 weeks until disease progression, unacceptable toxicity, or up to 24 months in patients without disease progression [see Clinical Studies (14.2)].

The recommended dose of KEYTRUDA is:

- 200mg for NSCLC that has not been previously treated with chemotherapy.
- 2mg/kg for NSCLC that has been previously treated with chemotherapy.

2.4 Recommended Dosage for Urothelial Carcinoma

The recommended dose of KEYTRUDA is 200 mg administered as an intravenous infusion over 30 minutes every 3 weeks until disease progression or unacceptable toxicity, or up to 24 months in patients without disease progression

2.5 Dose Modifications

Withhold KEYTRUDA for any of the following:

- Grade 2 pneumonitis [see Warnings and Precautions (5.1)]
- Grade 2 or 3 colitis [see Warnings and Precautions (5.2)]
- Grade 3 or 4 endocrinopathies [see Warnings and Precautions (5.4)]
- Grade 2 nephritis [see Warnings and Precautions (5.5)]
- Grade 3 severe skin reactions or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) [see Warnings and Precautions (5.6)]
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 and up to 5 times upper limit of normal (ULN) or total bilirubin greater than 1.5 and up to 3 times ULN
- Any other severe or Grade 3 treatment-related adverse reaction [see Warnings and Precautions (5.7)]

Resume KEYTRUDA in patients whose adverse reactions recover to Grade 0-1.

Permanently discontinue KEYTRUDA for any of the following:

- Any life-threatening adverse reaction (excluding endocrinopathies controlled with hormone replacement therapy)
- Grade 3 or 4 pneumonitis or recurrent pneumonitis of Grade 2 severity [see Warnings and Precautions (5.1)]
- Grade 3 or 4 nephritis [see Warnings and Precautions (5.5)]
- Grade 4 severe skin reactions or confirmed SJS or TEN [see Warnings and Precautions (5.6)]
- AST or ALT greater than 5 times ULN or total bilirubin greater than 3 times ULN

For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week

- Grade 3 or 4 infusion-related reactions [see Warnings and Precautions (5.8)]
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 adverse reactions (excluding endocrinopathies controlled with hormone replacement therapy) that do not recover to Grade 0-1 within 12 weeks after last dose of KEYTRUDA
- Any severe or Grade 3 treatment-related adverse reaction that recurs [see Warnings and Precautions (5.7)]

2.6 Preparation and Administration

Preparation for Intravenous Infusion

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.
- Dilute KEYTRUDA injection (solution) prior to intravenous administration.
- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.
- Discard any unused portion left in the vial.

Storage of Reconstituted and Diluted Solutions

The product does not contain a preservative.

Store the diluted solution from the KEYTRUDA 100 mg/4 mL vial either:

- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion solution in the IV bag, and the duration of infusion.
- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.

Do not freeze.

Administration

- Administer infusion solution intravenously over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.
- Do not co-administer other drugs through the same infusion line.

Side Effects:

Summary of the safety profile

Most common adverse reactions (reported in $\geq 20\%$ of patients) were fatigue, pruritus, diarrhea, decreased appetite, rash, dyspnea, constipation, and nausea. Additional adverse reactions reported in $\geq 20\%$ of patients with cancers other than melanoma and NSCLC were pyrexia, cough, and musculoskeletal pain.

Forensic Classification:

P1S1S3



Prepared by Lucilla Leung

Active Ingredient:

Elbasvir 50 mg / Grazoprevir 100 mg film-coated tablets

Presentation:

ZEPATIER 50 mg/100 mg filmcoated tablets

Pharmacological Properties:

Indications:

Therapeutic indications

ZEPATIER is indicated for the treatment of chronic hepatitis C (CHC) in adults.

For hepatitis C virus (HCV) genotype-specific activity.

Posology and method of administration

ZEPATIER treatment should be initiated and monitored by a physician experienced in the management of patients with CHC.

Posology

The recommended dose is one tablet once daily.

Recommended regimens and treatment durations are provided in **Table 1** below:

Table 1. Recommended ZEPATIER therapy for treatment of chronic hepatitis C infection in patients with or without compensated cirrhosis (Child-Pugh A only).	
HCV genotype	Treatment and duration
1a	ZEPATIER for 12 weeks ZEPATIER for 16 weeks plus ribavirin A should be considered in patients with baseline HCV RNA level $>800,000$ IU/ml and/or the presence of specific NS5A polymorphisms causing at least a 5-fold reduction in activity of elbasvir to minimise the risk of treatment failure
1b	ZEPATIER for 12 weeks
4	ZEPATIER for 12 weeks ZEPATIER for 16 weeks plus ribavirin A should be considered in patients with baseline HCV RNA level $>800,000$ IU/ml to minimise the risk of treatment

A In the clinical studies, the dose of ribavirin was weightbased (< 66 kg = 800 mg/day, 66 to 80 kg = 1,000 mg/day, 81 to 105 kg = 1,200 mg/day, > 105 kg = 1,400 mg/day) administered in two divided doses with food

For specific dosage instructions for ribavirin, including dose modification, refer to the ribavirin Summary of Product Characteristics.

Patients should be instructed that if vomiting occurs within 4 hours of dosing, an additional tablet can be taken up to 8 hours before the next dose. If vomiting occurs more than 4 hours after dosing, no further dose is needed.

In case a dose of ZEPATIER is missed and it is within 16 hours of the time ZEPATIER is usually taken, the patient should be instructed to take ZEPATIER as soon as possible and then take the next dose of ZEPATIER at the usual time. If more than 16 hours have passed since ZEPATIER is usually taken, then the patient should be instructed that the missed dose should NOT be taken and to take the next dose per the usual dosing schedule. Patients should be instructed not to take a double dose.

Elderly

No dose adjustment of ZEPATIER is required for elderly patients.

Renal impairment and end stage renal disease (ESRD)

No dose adjustment of ZEPATIER is required in patients with mild, moderate, or severe renal impairment (including patients receiving haemodialysis or peritoneal dialysis).

Hepatic impairment

No dose adjustment of ZEPATIER is required in patients with mild hepatic impairment (ChildPugh A). ZEPATIER is contraindicated in patients with moderate or severe hepatic impairment (ChildPugh B or C).

The safety and efficacy of ZEPATIER have not been established in liver transplant recipients.

Paediatric population

The safety and efficacy of ZEPATIER in children and adolescents aged less than 18 years have not been established. No data are available.

Contraindications:

Hypersensitivity to the active substances or to any of the excipients.

Patients with moderate or severe hepatic impairment (ChildPugh B or C).

Co-administration with inhibitors of organic anion transporting polypeptide 1B (OATP1B), such as rifampicin, atazanavir, darunavir, lopinavir, saquinavir, tipranavir, cobicistat or ciclosporin.

Co-administration with inducers of cytochrome P450 3A (CYP3A) or P-glycoprotein (P-gp), such as efavirenz, phenytoin, carbamazepine, bosentan, etravirine, modafinil or St. John's wort (*Hypericum perforatum*).

Precautions:

The rate of late ALT elevations during treatment is directly related to the plasma exposure to grazoprevir. During clinical studies with ZEPATIER with or without ribavirin, < 1 % of subjects experienced elevations of ALT from normal levels to greater than 5 times the upper limit of normal (ULN). Higher rates of late ALT elevations occurred in females (2 % [11/652]), Asians (2 % [4/165]), and subjects aged ≥ 65 years (2 % [3/187]). These late ALT elevations generally occurred at or after treatment week 8.

Hepatic laboratory testing should be performed prior to therapy, at treatment week 8, and as clinically indicated. For patients receiving 16 weeks of therapy, additional hepatic laboratory testing should be performed at treatment week 12.

- Patients should be instructed to consult their healthcare professional without delay if they have onset of fatigue, weakness, lack of appetite, nausea and vomiting, jaundice or discoloured faeces.

- Discontinuation of ZEPATIER should be considered if ALT levels are confirmed to be greater than 10 times the ULN.

- ZEPATIER should be discontinued if ALT elevation is accompanied by signs or symptoms of liver inflammation or increasing conjugated bilirubin, alkaline phosphatase, or international normalised ratio (INR).

Drug Interactions:

Coadministration of ZEPATIER and OATP1B inhibitors is contraindicated because it may significantly increase grazoprevir plasma concentrations.

Co-administration of ZEPATIER and CYP3A or P-gp inducers is contraindicated because it may significantly decrease elbasvir and grazoprevir plasma concentrations and may lead to a reduced therapeutic effect of ZEPATIER.

The concomitant use of ZEPATIER and strong CYP3A inhibitors increases elbasvir and grazoprevir concentrations, and co-administration is not recommended.

Side Effects:

Summary of the safety profile

The safety of ZEPATIER was assessed based on 3 placebocontrolled studies and 7 uncontrolled Phase 2 and 3 clinical studies in approximately 2,000 subjects with chronic hepatitis C infection with compensated liver disease (with or without cirrhosis).

In clinical studies, the most commonly reported adverse reactions (greater than 10%) were fatigue and headache. Less than 1 % of subjects treated with ZEPATIER with or without ribavirin had serious adverse reactions (abdominal pain, transient ischaemic attack and anaemia). Less than 1 % of subjects treated with ZEPATIER with or without ribavirin permanently discontinued treatment due to adverse reactions. The frequency of serious adverse reactions and discontinuations due to adverse reactions in subjects with compensated cirrhosis were comparable to those seen in subjects without cirrhosis.

When elbasvir/grazoprevir was studied with ribavirin, the most frequent adverse reactions to elbasvir/grazoprevir + ribavirin combination therapy were consistent with the known safety profile of ribavirin.

Tabulated summary of adverse reactions (see **Table 2**)

The following adverse reactions were identified in patients taking ZEPATIER without ribavirin for 12 weeks. The adverse reactions are listed below by body system organ class and frequency. Frequencies are defined as follows: very common (≥ 1/10), common (≥ 1/100 to < 1/10), uncommon (≥ 1/1,000 to < 1/100), rare (≥ 1/10,000 to < 1/1,000) or very rare (< 1/10,000).

Table 2. Adverse reactions identified with ZEPATIER*
Frequency Adverse reactions
Metabolism and nutrition disorders: Common decreased appetite
Psychiatric disorders: Common insomnia, anxiety, depression , Nervous system disorders: Very common headache, Common dizziness
Gastrointestinal disorders: Common nausea, diarrhoea, constipation, upper abdominal pain, abdominal pain, dry mouth, vomiting
Skin and subcutaneous tissue disorders: Common pruritus, alopecia
Musculoskeletal and connective tissue disorders: Common arthralgia, myalgia
General disorders and administration site conditions: Very common fatigue, Common asthenia, irritability

*Based on pooled data from patients treated with ZEPATIER for 12 weeks without ribavirin.

Forensic Classification:

P1S1S3

關節急救 一盒見效

[1-6]

服用蛋膜素效果比
葡萄糖胺、軟骨素
功效顯著多達

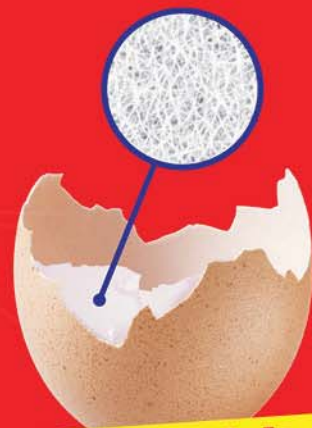
5 倍^[6]

- 臨床實證，天然蛋膜素能於**7-10天**內有效緩解關節疼痛和關節僵硬情況^[1-6]
- 小分子酶解技術，人體吸收更快更有效
- 適合關注關節健康、關節疼痛、頸腰椎勞損、長期運動及骨傷手術後人士



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 (5) Clegg DO, et al., N Engl J Med 2006, 354(8):795-808
 (6) NNT for glucosamine and chondroitin calculated from the GAIT study (N Engl J Med 2006, 354(8):795-808)(1) Kevin J. Ruff, et al., Food and Chemical Toxicology 2012,(50): 604-611
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甚麼是蛋膜素?

蛋膜是蛋殼內側厚度約 0.07~0.1mm 的一層薄膜。構造為緊密的纖維網狀，保護雞蛋免受外部衝擊。

蛋膜經過先進獨特的酶解技術萃取後成為**天然蛋膜素** (又稱關節靈活素)。

天然蛋膜素當中包括了**骨膠原** (I, V, X型)，**肽**、**氨基酸** (甲硫氨酸、半胱氨酸)等蛋白質，還含有**葡萄糖胺**、**軟骨素**、**皮膚素**、**角質素**、**透明質酸**等，這些都是補充關節健康的必需營養。就如同伸展的**緩衝膜**，**鞏固關節**。

臨床驗證^[4-5] 功效顯著 服食前後分別



- 科研創健 · 活樂人生 -

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