News & Short Communications

The Use of Biologics (Anti-TNF) in Immunologic Diseases (2 CE Units)

Absence of Chlorogenic Acid in *Ginkgo biloba* Leaf Samples and Their Over-the-Counter Products

Preparative Separation, Characterization, and Determination of Major Compounds in *Gynostemma pentaphyllum* Using Two-dimensional Counter-current Chromatography and Information-dependant Acquisition Mediated UPLC–MS/MS

Bioactive Substances and Medicinal Effects of Lycii Cortex

Pharmacy Study and Research Tour with China Pharmaceutical University

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Publish or Perish Paranoia

Nowadays, many people in the academe consider publication in the SCI (Science Citation Index) journals as something that is really precious and extraordinary while condemning publication in the local or regional journals as lacking merits and rubbish. This type of belief might be perpetuated by the meritoric “hype” of the real score assigned by some publishers. In fact, many articles are actually trash, yet they are published. Just to ensure publication, some editors may contact a friend or a colleague that knew a little or nothing at all about the topic in order to maintain a facade of a peer review. Many low impact factor journals are starving for articles to be published and in this case, the “beggars can’t be choosy” strategy may apply. In another scenario, racism, bias against views or conflict of interest may overrule the fair implementation of the review procedure. Thus, the basis for rejection of a publication may be disputable. It is also a little bit flimsy to tap a doctorate degree holder in a particular field without a significant track record about the experimental design of the manuscript they are asked to review. The manuscript should be rated based on its merits to that particular field. After a rigorous peer review, the editors should do an impartial judgment of the manuscript at the same time rather than solely depending on the referees’ decision. In the real scenario, however, there are still many institutions and companies around the world that do not make publication a big deal or even make it as a classic archetype for something. In fact, many employees got promoted in their own way even without SCI publication. Actually, when we call a journal to be SCI, it simply informs us of its existence.

Even though, there is expediency for academicians and professionals to publish in an international peer reviewed SCI journals, one should remember that it does not always guarantee quality of performance. It is quite hilarious but it is a common practice that many authors or co-authors are just listed due to gratitude because they donated or sponsored something, that they provided a lab to work on, and that maybe for the sake of friendship even without a thorough glimpse of the experimental design or even without the capability of framing a research paper. Even worst, some may fabricate data, plagiarize other people’s work or even employ non-scientific and non-acceptable research procedures. Yet their papers got published in the so called SCI journals. Just figure it out how a circle of experts constantly validating, corroborating, citing, and reviewing each other’s articles. Also, one should note that there are journals that are less stringent in accepting articles than the others yet they are SCI. The imprudent immortalization of SCI journals is usually coupled with the apparent humiliation of local journals that they are cursed to produce garbage papers. Thus, one should consider these a posteriori realizations over and above glorification of SCI-indexed journal publication while discriminating non-SCI-indexed journals a priori.

In the academe, the acknowledgment for superb teaching is seldom equally appreciated to the acknowledgment given to exemplary research. In fact most universities simply focus on the publication lists and the ability to earn research money rather than focus on competency of teaching a specific area of knowledge upon hiring a new faculty. Besides, most research universities often considered classroom mentoring as a secondary position. This narrow minded focus on the faculty as a researcher may result in negligence of other functions and dedication to effective teaching. The essence of mentorship may be lost especially if the students are misguided by simply assigning incapable graduate students whose expertise are different from the subject being taught and they are even incompetent enough to evaluate the students’ progress. Furthermore, some academicians and scientists put so much emphasis on publishing that instead of thinking new research agenda, they spend so much time on reshuffling and refurbishing their data just for the sake of publication. In fact, the cause of poor articles being submitted to academic journals may be that some people merely push to publish in order to sustain one’s tenure.

However, the drive for SCI publication is a relative trend in prestigious universities in Asia including Hong Kong. Some universities may now give cash incentives and even grant promotion and tenure to their faculty and staff. In some cases, a publication is considered a prerequisite for a PhD degree. Besides, SCI publication can be a conducive tool for executives and funding agencies to find out what really matters. Usually, a journal, no matter considered publications as one of the basic criteria for ranking university performance. Even in the field of Pharmacy, pharmacists are encouraged to publish in SCI journals. Somewhat, we hope that HKPJ will be consideredSCI in the future. Nonetheless, whether HKPJ will be considered as SCI in the future or not, if the hurdle for publication is lax, then it is still vulnerable to becoming another rubbish bin for scrap articles. As pharmacists, we should develop and encourage a community of exchanging views through prolific journal discussion and critiquing. Furthermore, we can think of ways of filling the gaps for the future advancement of pharmacy as a science.

In the past years, many pharmaceutical researches and clinical studies had been done to discover new drugs and to alleviate human illnesses. The meta-analysis done by Dr. Douglas G. Adler and colleagues found out that there is sufficient evidence that proton-pump inhibitors (PPIs) increase the incidence of Clostridium difficile associated diarrhea (CDAD). On the other hand, they further concluded that establishing a guideline for the use of PPIs may help in the future with the judicious use of PPIs. They suggested further prospective studies to fully explore the association between PPIs and CDAD (p.97). Recently, new drugs or drug combinations were approved by FDA to treat advanced breast cancer, osteoarthritis, diabetes, and obesity (p. 97-100). However, problems do arise because half of the heart patients do not stick to their meds (p.98). In Canada for instance, long term use of calcium containing drugs poses a cancer risk and serious side effects are more likely in new cancer drugs (p.98). Winter is approaching in the coming months. Common coughs and colds are usually associated with this climatic change. Consumers and patients including parents of children are advised to consider possible safety risks for the common cold and cough applications (p.99).

The use of biologics in the treatment of chronic inflammatory diseases has been presented by Tse and Luk in the Drugs and Therapeutics Section (p.102). However, safety concerns have been raised with regards to its use (p.105). Nonetheless, the development of this research explores other components in the inflammatory pathway as a room for further research. In the Over-the-Counter and Health Section, Zhu et al. discovered that from both TLC assays and HPLC analysis, another compound present in Ginkgo Folia exhibits similar TLC behaviour after heating to chlorogenic acid, and is in fact the real marker in TLC analysis. Further LC/MS/MS analysis of the compound, previously mistaken to be chlorogenic acid, revealed that it is 6-hydroxyxynurenic acid, which is one of the many ingredients reported in Ginkgo leaves. Hence, they recommended that 6-hydroxyxynurenic acid, instead of chlorogenic acid, should be properly adopted as the marker for quick TLC identification of the Ginkgo leaf and its derived functional food. The discovery of this research work has been endorsed in year 2009 by the Scientific Committee and the International Advisory Board of the Hong Kong Chinese Materia Medica Standards (p.111). Another study by Wang and Cheung presented in the Pharmaceutical Technique and Technology Section, a method for preparative separation, characterization and determination of rutin, isorhamnetin-3-O-β-D-glucopyranoside, isorhamnetin and cirsiliol from Glyostemma pentaphyllum was successfully established (p.112). The results showed that EECGC coupling with IDA−UPLC−MS/MS is an efficient way for separation, characterization and determination of major compounds from natural sources. Modern pharmacological studies revealed that extracts of isorhamnetin-3-O-β-D-glucopyranoside obtained from Lycii cortex have many biological activities including antihypertensive, anti-diabetic, anti-inflammatory, antioxidant, antibiotic, and anti-parasitic. Hence, it is worthwhile to develop and explore this Chinese Materia Medica according to Zhang and his colleagues (p.120). Above all these printed information, knowledge and skills require frequent exchange amongst the professionals. Thus, both students and staff are encouraged to exchange their knowledge and experience in clinical practice, teaching and research, to foster future partnership and collaboration (Chan et al., p. 124).

Lastly, whether we publish more or less paper or none at all, will not make us the least nor the best person in the pharmacy profession. To have an article published may be an accessory to the development of one’s career but it may not be the ultimate criteria considering the significant contribution to the field. However, a journal, no matter categorized as SCI or not, is a way of documenting or exchanging relevant knowledge and information that could be beneficial to the maintenance of health of the future generation. Hence, non-SCI publication should not be totally condemned and ignored.

Cheung Hon-Yeung
Editor-in-Chief
17 October 2012
**News & Short Communications**

**PPI Therapy Tied to Clostridium-difficile-Associated-Diarrhea in Meta-Analysis**

**Date: June 19, 2012**

According to Dr. Douglas G. Adler and colleagues, there is sufficient evidence that proton-pump inhibitors (PPIs) increase the incidence of *Clostridium difficile*-associated diarrhea (CDAD). They recommend that the routine use of PPIs for gastric ulcer prophylaxis should be more prudent. Dr. Yoon K. Loke, of Norwich Medical School, University of East Anglia, Norwich, United Kingdom agrees. He said that many patients (usually elderly) are on PPIs long-term, and doctors are unable to work out why they were started on the PPI in the first place, and when the treatment should be stopped. Patients seem to get left on these drugs because healthcare professionals aren’t aware of the harm.

CDAD is a major cause of illness — and an expensive one, with a price tag estimated at 3 billion US dollars annually. Antibiotic use remains the dominant risk factor for CDAD; other risk factors include advancing age, severe underlying illness, hospitalization, use of nasogastric tubes, antineoplastic chemotherapy and immunosuppressants. More recently, PPI use has garnered attention as a risk factor.

Dr. Adler’s team searched Medline and PubMed for studies that investigated the association between PPIs and CDAD from 1990 to 2010. They ultimately included 23 studies involving close to 300,000 patients. According to the authors, the summary risk ratio for CDAD among patients on PPIs was 1.69 (p<0.000). Whether case-control study (17 studies) or cohort study (six studies), a significant increase in the incidence of CDAD among PPI users was seen. The risk estimates were 2.31 (p< 0.001) and 1.48 (p< 0.001) for cohort and case-control studies, respectively.

Dr. Adler and colleagues conclude: “Establishing a guideline for the use of PPI may help in the future with the judicious use of PPIs. Further studies, preferably prospective, are needed to fully explore the association between PPIs and CDAD. These studies should focus on evaluating the dosage and duration of PPI use and the risk of CDAD and its recurrence.”

Source: Am J Gastroenterol. Published online June 19, 2012.

**Australia: Why Hasn’t The TGA Taken Stilnox Off The Market**

**Date: July 7, 2012**

TGA stated that Stilnox containing zolpidem is a medicine of value to some patients if used properly, particularly with severe insomnia, and should remain available for use in Australia while there is evidence of some extreme side effects in some people. According to TGA, the following warning was already highlighted in the product information of zolpidem: “Zolpidem may include associated with potentially dangerous complex sleep-related behaviours which may include sleep walking, sleep driving and other bizarre behaviours. Zolpidem is not to be taken with alcohol. Caution is needed with other CNS depressant drugs. Limit use to four weeks maximum under close medical supervision.”

In Hong Kong, zolpidem is prescription only medicine. Zolpidem is also controlled as psychotropic substances internationally. Warning of “Complex sleep-related behaviours” is already a labelling requirement for those products.


**FDA Approves New Diet Drug: Phentermine-Topiramate Combo**

**Date: July 17, 2012**

The FDA has approved the weight-loss drug Qsymia (formerly named Qnexa; Vivus, Mountain View, CA), which now joins lorcaserin (Belviq, Arena Pharmaceuticals, San Diego, CA) as the first anti-obesity medications to enter the US market since 1999.

Qsymia, a controlled-release preparation of phentermine and topiramate in one capsule, is now indicated for use in adults with a body mass index (BMI) >30 kg/m2 or adults with a BMI of >27 kg/m2 and at least one weight-related condition such as hypertension, type 2 diabetes, or dyslipidemia.

The approval is accompanied by a Risk Evaluation and Mitigation Strategy (REMS), including a medication guide advising patients about important safety information and specific requirements for prescriber training and pharmacy certification. The drug “will only be dispensed through specially certified pharmacies,” according to the FDA statement. Of special note, the drug must not be used during pregnancy; details about the risk of the drug combo in pregnant women are included.

The agency’s statement also notes that the drug can increase heart rate, which warrants regular monitoring, and should be used with caution in people with recent unstable heart disease or stroke. The sponsor will be required to conduct a long-term, postmarketing cardiovascular outcomes trial to assess the effect of Qsymia on the risk of major adverse cardiac events.

Source: Medscape Medical News
Canada: Calcitonin-containing drugs: Health Canada assessing potential cancer risk with long-term use

Date: July 23, 2012

Health Canada announced that it is assessing the possibility of an increased risk of cancer with long-term use of the drug calcitonin. Calcitonin is a prescription drug available in Canada as a nasal spray used to treat osteoporosis in postmenopausal women. It is also available as a solution for injection used to treat Paget's disease as well as severe hypercalcemia.

Health Canada is aware of new recommendations from the European Medicines Agency (EMA) to restrict calcitonin use due to evidence suggesting an increased risk of cancer. Specifically, the EMA has recommended that 1) calcitonin nasal spray should no longer be prescribed for the treatment of osteoporosis; 2) injectable calcitonin should be used to treat patients with Paget's disease only when other treatments have not worked or are not appropriate, and treatment should normally be limited to three months; 3) injectable calcitonin should only be used for hypercalcemia caused by cancer.

Half of Heart Patients Don’t Stick to Their Meds

Date: July 25, 2012

According to a new review of several studies, just half of people who are given a prescription to prevent heart disease continue to get their medications refilled over time. The studies looked at seven medications, including aspirin, blood pressure drugs, and statins, typically intended for life-long use. Data from 20 studies suggested the rate at which people continue taking the drugs ranges from 30% to 80%.

Among people who have already had a heart attack, one out of every three fails to continue filling their prescription refills. “Even if these estimates were half as great, the cost of nonadherence is substantial,” the group, led by Dr. David Wald at the University of London, writes. The researchers estimated in their report online June 27 in the American Journal of Medicine that 130,000 people die each year because they don’t adhere to their medications.

Of the more than 376,000 people in the studies, about 275,000 were given a prescription to prevent heart disease while the other 101,000 were already diagnosed with heart disease. All of the patients were followed for at least 12 months. Overall, 57% of people continued to refill their prescriptions for the drugs.

“This is something that’s been going on for decades and we’ve been well aware,” said Dr. David Blackburn, an associate professor of pharmacy and the research chair in patient adherence at the University of Saskatchewan in Canada, who was not involved in the research. Dr. Blackburn said there are few interventions known to consistently help patients stay on track with their medications, because it’s not entirely clear why they’re not compliant. In some cases, it could be related to the patient - difficulty reading the drug label or opening the container, fear of side effects, or challenges making it to the pharmacy for a refill.

The health care system could play a role, too. “It’s difficult to have a real discourse with a physician because... everybody’s busy,” Dr. Blackburn said. “Because of the system and the constraints on cost and time, I think what you end up with are people who are really inadequately prepared” to follow through with their prescriptions.

For the most part, patients complied with their prescriptions at about the same level for each of the different types of drugs. The only difference was among people without a diagnosis of heart disease, who were less likely to continue taking diuretics than they were to continue on angiotensin receptor blockers. “This suggests that specific drug properties (such as how often people have to take them or side effects) have a minor influence on whether patients remain on treatment long-term,” the authors write.

Dr. Blackburn said the findings support his ideas of what causes people to drop off of their medications. “It’s probably system-related factors that are so important that they dwarf these little tolerability issues. They get drowned out by the way prescriptions are given and the time we have to engage with people,” he said. He thinks frequent follow-ups with patients to make sure they’re continuing their medication is important to help people stay on track.


Combination Type 2 Diabetes Pill Approved in Europe

Date: July 27, 2012

The European Commission has approved the combination pill containing the dipeptidyl peptidase-4 inhibitor linagliptin and metformin hydrochloride for the treatment of adults with type 2 diabetes, alongside diet and exercise. The approval follows a favorable recommendation by the European Medicines Agency in May. The linagliptin/metformin combination sold in Europe as Jentadueto (Boehringer Ingelheim Pharmaceuticals, Inc, and Eli Lilly & Co) provides a single-tablet option that is taken twice daily.

It is intended for patients with type 2 diabetes that is inadequately controlled on their maximal tolerated dose of metformin alone, metformin plus a sulfonylurea, or those already being treated with the combination of linagliptin and metformin. It can be used alone or in combination with a sulfonylurea. The linagliptin/metformin combination was approved by the US Food and Drug Administration earlier this year.

In a 24-week, randomized, double-blind, placebo-controlled study involving 791 adults with type 2 diabetes inadequately managed with diet and exercise, 2.5 mg linagliptin/1000 mg metformin led to mean reductions in hemoglobinA1c levels of 1.7 percentage points. Statistically significant reductions in fasting plasma glucose (FFPG) of -60 mg/dL were also seen. In clinical studies, linagliptin/metformin did not cause any significant change in body weight. Adverse reactions were uncommon in clinical studies. Gastrointestinal disorders occurred most often during the initiation period and tended to resolve spontaneously. A comparable rate of diarrhea was reported with linagliptin/metformin treatment vs metformin plus placebo. Hypoglycemia was more commonly reported in patients treated with the combination of linagliptin/metformin and a sulfonylurea compared with those treated with the combination of placebo, metformin, and a sulfonylurea. The linagliptin/metformin combination pill is not indicated for patients with type 1 diabetes or those who have diabetic ketoacidosis. It has not been studied in combination with insulin. Linagliptin/metformin combination tablets will be made available in the following twice-daily doses in Europe: 2.5 mg linagliptin/850 mg metformin tablets and 2.5 mg linagliptin/1000 mg metformin tablets.

Source: Medscape Medical News
Everolimus Approved for Advanced Breast Cancer in Europe & US

Date: July 31, 2012

The European Commission has approved everolimus (Afinitor, Novartis) for use with exemestane (Aromasin, Pfizer) to treat postmenopausal women with advanced breast cancer, according to a Novartis Oncology press release. The indication is for the treatment of hormone-receptor-positive, HER2-negative breast cancer after progression on an aromatase inhibitor. The US Food and Drug Administration has also approved everolimus for the same indication 2 weeks earlier.

The approval was based on the phase 3 Breast Cancer Trials of Oral Everolimus-2 (BOLERO-2). In that randomized multicenter study of 724 patients, local investigator assessment showed that the addition of everolimus to exemestane more than doubled median progression-free survival — to 7.8 months from 3.2 months with exemestane alone (hazard ratio, 0.45; 95% confidence interval, 0.38 to 0.54; P < .0001). Another analysis, based on independent central radiology, also showed that the combined treatment extended median progression-free survival — to 11.0 months from 4.1 months with exemestane alone (hazard ratio, 0.38; 95% CI, 0.31 to 0.48; P < .0001).

The most common grade 3/4 adverse reactions (incidence of 2% or more) were stomatitis, infections, hyperglycemia, fatigue, dyspnea, pneumonia, and diarrhea.

“Everolimus is the most important advance in breast cancer since trastuzumab,” said Fabrice André, MD, from the Institut Gustave Roussy, Paris, France, when the results of a preplanned interim analysis of BOLERO-2 were reported at the 2011 European Multidisciplinary Cancer Congress. However, when updated findings from the trial were reported later in 2011 at the 34th Annual San Antonio Breast Cancer Symposium, another expert had some more tempered comments about the drug. The rate of response to the combination therapy in BOLERO-2 was low, at 12%, said Harold Burstein, MD, from the division of breast oncology at the Dana-Farber Cancer Institute in Boston, Massachusetts, who was not involved in the study. However, coprincipal investigator of BOLERO-2, José Baselga, MD, from the Massachusetts General Hospital and Harvard Medical School in Boston, said that “Everolimus is the first agent to enhance hormone therapy in refractory ER-positive breast cancer patients,” and the results of the trial represent a “paradigm shift in the management of these patients.”

Source: Medscape Medical News

Australia: Children’s cough and cold medicines - TGA advice

Date: August 15, 2012

The TGA has conducted a review of the use of cough and cold medicines in children. The review concluded that there are no immediate safety risks with these products. However, there is evidence that they may cause harm to children. Furthermore, the benefits of using them in children have not been proven. On this basis, TGA advised that cough and cold medicines should not be given to children under 6 years of age and should only be given to children aged 6 to 11 years on the advice of a doctor, pharmacist or nurse practitioner. The labels of these products are being changed in Australia to reflect the new advice. Stock with the new labelling will begin to appear in pharmacies and other retail stores from September 2012.

In Hong Kong, the Registration Committee of Pharmacy and Poisons Board had decided in April 2009 that pharmaceutical products for the treatment of cough and cold should not be used for children under 6 years of age, and the label of cough and cold products should be revised accordingly.


Topical NSAIDs May Be a Better Choice for Elderly With OA

Date: September 18, 2012

Topical diclofenac is about as effective as oral diclofenac in knee and hand osteoarthritis (OA), is probably as effective as other oral NSAIDs, and might be a safer choice for elderly patients and others at risk for gastrointestinal adverse effects. Sheena Derry, PhD, and colleagues from the University of Oxford in the United Kingdom based their conclusions about topical NSAIDs on a review of randomized, double-blind studies with placebo or active comparators in which at least a single treatment was a topical NSAID used to treat chronic pain caused by OA, and in which treatment lasted at least 2 weeks. The analysis included data from 7688 participants in 34 studies, 23 of which compared a topical NSAID with placebo.

“Topical NSAIDs were significantly more effective than placebo for reducing pain due to chronic musculoskeletal conditions,” the authors conclude. “Direct comparison of topical NSAID with an oral NSAID did not show any difference in efficacy. Topical NSAIDs were associated with more local adverse events, such as mild rash, but with fewer gastrointestinal adverse events than oral NSAIDs”.

For topical diclofenac, the number needed to treat (NNT) for at least 50% pain relief vs placebo was 6.4 for diclofenac solution and 11 for diclofenac gel formulation. There were insufficient data to calculate NNTs for other individual topical NSAIDs. “The results presented here show clearly that high quality large studies demonstrate efficacy of topical NSAIDs in 12 week studies, with NNTs similar to those of oral NSAIDs,” the authors write. Experimental data suggest that creams are generally less effective than gels or sprays, according to the authors. “One of the features of topical NSAIDs is that formulation has the potential to play a big part in efficacy,” Dr. Moore said.

The authors wrote that it is probable that topical NSAIDs exert their action both by local reduction of symptoms arising from periartricular structures, and by systemic delivery to intracapsular structures. Tissue levels of NSAIDs applied topically certainly reach levels high enough to inhibit cyclooxygenase-2. Plasma concentrations found after topical administration, however, are only a fraction (usually much less than 5%) of the levels found in plasma following oral administration. Topical application can potentially limit systemic adverse events by increasing local effects, and minimizing systemic concentrations of the drug. The upper gastrointestinal bleeding is low with chronic use of topical NSAIDs.

Source: Database Syst Rev. Published online September 12, 2012. Abstract
食物環境及衛生局局長高永文醫生談香港未來中醫藥發展

前言

新政府上場，著名骨科醫生高永文，亦搖身一變成食物環境及衛生局局長。以下
是高醫生在上任前跟大家談香港未來中醫藥發展的問題。他表示他個人對中醫藥
的最大心愿，是令中醫可以和西醫齊齊，
同樣可以有中醫診療服務，甚或成立中醫
院。

香港中醫藥的發展

香港的中醫藥發展不是這一刻的事，而是
很多歷史的總和。和高永文談香港中醫藥
的未來之前，他娓娓道來香港中醫藥的歷
史。從1950年代中醫在學校裡被抽出，
港英政府成立工作小組，研究中醫在香港
的情況；到1960年代開始對中醫進行評
估，同時三間大學，陸續開始提供全制中
藥學課程；至1970年代，正式通過行政
會議撥款，醫療局在十八區公立醫院體
系中成立中藥藥房和研究中心，向市民
直接提供中藥藥品，而模式則由志願組
織、學術機構及醫院三方合作管理。

著重中醫培訓

高永文多年來一直主張中西醫合璧。擔任
食物及衛生福利局局長後，中醫藥將如
何在西醫為主導的香港活下去，是他將來
的一大挑戰。他說，未來的工作，將會集
中於中醫培訓。「首先，我們的中藥藥學
生畢業出來，在培訓上為之辛苦，因臨床
培訓上中藥學作為專業，很多地方都是一
地。中藥畢業生的發展空間也有限，現在
只有各區醫院的臨床研究中心提供培訓和
就職機會。」

他續說，即使現在不少醫院都有中醫
診症，不過仍未完善。「服務方面，現在
時仍停留在臨床門診，住院服務仍然未有，
因病人仍是西醫主診，就算有某些醫
院提供有限的中醫治療，病人都需要到
西醫同意，才可邀請院內中醫藥中心的
中醫過來會診。在這情況下，業界有訴
求，而特首梁振英在選舉政綱中亦作出回
應。」

成立中醫康復醫院

說到培訓，中醫院絕對是培訓的好地方，
也是病人一直爭取多年的願望。高永文
說，這個心願亦有於幾年來落實，不過最
初可能不是全科醫院，而是康復醫院。

「當然講緊的中醫院並不是純中醫院，
或像內地般的中西醫結合的醫院，當中
中醫可開西藥，西醫開中藥。因為以香港
的專業機構架構，暫時未必能接納。」
欲找個適合香港的模式，因此香港的中
醫院，將來是有中醫特色或中醫主導的醫
院。」

所指「中醫主導的醫院」，是指真正
的中醫住院服務，從病人入院起全部
中醫，或中醫負責。高永文指出，這
樣做對臨床研究和臨床教學會較為方便。
「因為很多時臨床研究，都要醫院做
基。此外，市民亦可有多個中醫藥服務
的選擇。而現時，除了國內，在中國內的
地方就只有德醫有類似的中醫院，是由中
國國家中醫藥管理局協助成立，已有十多
年歷史。」

「我想這是向估 América 機施的，特
會研究這個可能性。」他說，中醫院牽
涉很多問題，需要一一解決。「將來要搞
得中，能否做到？多久才能做到？實行起
來如何？運營可行性如何？運作模式怎樣？
定位如何？通通都要研究的。」

中醫藥將服務三類病人

高醫生認為，中醫主導的醫院，定位很
重要，因為有定位，才能持續發展。
「然而，要幫香港中醫院找出定位，要很
小心。因為香港病人通常有重病、急病才
入院。那麼什麼樣的病人才有興趣入院接
受中醫的服務？我想先要找出這些主要
病類。」而高醫生個人認為，有以下三類
病類，較需要中醫醫院的服務：

(1) 中風或長期康復病人
「因為現在公立醫院康復病床不足，很多時
會鼓勵病人在家康復，但家中康復有其
限制。然而陸續有中醫，中風康復病人
經中鋼醫院治療，跟著幾個月，中風病
的康復，是否可以在中醫醫院內進行？」

(2) 癌症病人
並非所有癌症病人，而是接受放療、化
療，有副作用的病人。「醫院主要以日間
服務形式提供這兩種服務，病人通常不用
住院，但偏性化療放療的病人會出現很多
副作用，他們是否可以去中醫院？因中醫
藥很多都能抗化療、放療的副作用。此
外，較嚴重的癌症病人、長期癌症病人、
身體情況不是太好的病人，較適合在香港
醫院內有這些治療的癌症復病人，或接
受中西醫藥學治療的病人，是否亦較適合
在中醫醫院內治療呢？」

(3) 嚴重病症
「行動不方便的，可否在中醫醫院接受一個
短期的治療呢？」

但高醫生強調：「以上全屬個人意見，還
要待明日一齊商討，中醫藥局服務定位是
怎樣？定位好緊要，這樣才可以講可持續
性。」

中藥發展不能急

說到中藥，當然要家中藥。對於中藥的
發展，高永文認為正面對很多難題，絕對
不能心急。高永文醫生坦言：「這十多年
以來，逢人問我如何發展中藥產業，我都
說不能急。大家要明白，中藥或中成藥作
為醫療產業的一部份，不是一蹴而就，而
是安全性問題，至今仍未完全完成。因
為中藥的規格，特別中成藥的規格，要非
常嚴格，否則保障低。但大夥新西方的
標準，又會窒息中藥產業的發展。」

他說，光是要求中藥的認可、可重複
性和穩定性已產生很多問題，不易解決。
還有療效的臨床研究，若一百百分按西方
的黃金標準去做，則不受科學界接受。但
為了證明雙盲對照，隨機對照，結果中藥
的調配就需轉變形式，例如以藥丸和
藥粉發放給病人服用。此外，不只中醫
藥，就是中藥發展，因為租金、成本
高，臨床研究目前未有很大的基礎，所以
中藥產業的發展是否一定要跟隨中醫服務
發展也成為一個問題？所以，我說要解決
中藥發展不能急。」

幸好，西方國家最近開始明白到，未
必要在細胞層面去追求單一種藥物的機理，
而是所謂的「多靶點治療」。高醫生說：
「是指人的機能複雜，不是單純看一種藥
物的治療成效，而是看一種藥可有不同的
功能區域及細胞內不同的靶點，會更有利
治療。其實變相跟中藥的治療理論接近，
對中藥邁向世界也有一定好處。」

成立發展委員會

最後，高永文醫生說，為了對應市民和業
界對中醫藥發展的需求。新政府上場，
將考慮成立中醫藥發展委員會，或中醫藥
發展委員會，認為宜去發展本地的中藥。」

「成立發展委員會的目的，就是要將這推進
本地中藥發展，亦表明目前的政策方向
會較圍繞性，更全面而有力。而且，成立委
員會亦將可有力地協調不同政策範圍，配
合中藥業的發展。」
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Gastro-intestinal lesions caused by endoscopy or radiation therapy

- Double bio-adhesion of sucralfate cyto-protective layer to damaged, inflamed G.I. mucosae
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Laboratorio Italiano Biochimico Farmaceutico
LISAPHARMA S.p.A.
22036 Erba (Como) - Italy

Mekim Limited
Enquiry: 2774 8385
The Use of Biologics (Anti-TNF) in Immunologic Diseases

TSE, Kit-Yinga; LUK, Stella*b

a Pamela Youde Nethersole Eastern Hospital, 3 Lok Man Road, Chai Wan, Hong Kong SAR, China;
b Pfizer Corporation Hong Kong Ltd., 16/F, Stanhope House, 736 King’s Road, North Point, Hong Kong SAR, China (*Corresponding author)

ABSTRACT

Tumor necrosis factor antagonists (anti-TNF agents) are biologics with documented efficacy in the treatment of chronic inflammatory diseases. There are mainly three types of anti-TNF agents available in Hong Kong – infliximab, adalimumab and etanercept. They have different structures and TNF-binding characteristics, which may possibly lead to difference in efficacy and safety profiles of the three agents. Anti-TNF agents may induce neutralizing antibody formation which may reduce the efficacy of the agents in a long run. Recent clinical development explores other components in the inflammatory pathway to work on. In some overseas country, there is special requirement for the registration of biologics.

Keywords: Biologics, immunologic diseases, tumor necrosis factor, anti-TNF agents, TNF antagonists

INTRODUCTION

What is biologics?

In a broad sense, biologics (or biological products) refer to a wide range of medicinal products which are derived from various natural resources, including human, animal or microorganism, and may be produced by biotechnology methods. Examples of biologics include blood components, allergens, vaccines, tissues, immunoglobulin products and recombinant therapeutic proteins. (1)

However, the term “biologics” is often used more restrictively for genetically engineered proteins that are derived from human DNA. (2) In such context, biologics can be substances that are nearly identical to the key signaling proteins of the human body, such as biosynthetic human insulin and its analogues; (3-4) fusion proteins which are based on naturally-occurring receptor of certain human immunoglobulins; (5-6) or monoclonal antibodies which have specific structure to block targeted substances or cell types in the body. (7-9) In recent years, many biologics have been developed to counteract the inflammation, which occurs in various immunological diseases in the field of rheumatology, dermatology and gastroenterology, etc.

DISCUSSION

Common immunologic diseases and immunosuppressive drugs

Rheumatoid arthritis (RA) is one of the most frequently encountered immunologic diseases. The conventional therapies include non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants, which include corticosteroid and disease modifying anti-rheumatoid drugs (DMARDs) such as methotrexate. (10) However, existing drug options are often inadequate to properly manage the disease state. Their toxicities may also limit their use. Therefore, despite aggressive management with oral immunosuppressive agents, biologics is now an additional option to the existing therapies.

Unlike conventional immunosuppressants which broadly suppress the whole immune system, biologics selectively block the effects of a particular component in the inflammatory pathway. For example, tumor necrosis factor (TNF) antagonists, such as infliximab, adalimumab and etanercept, primarily block the interaction between TNF (previously known as TNF-α) and their receptors on the surface of immune cells. (11) These tumor necrosis factor antagonists, also known as anti-TNF agents, are novel agents which have successfully demonstrated efficacy in managing immunologic diseases.

Anti-TNF agents

Tumor necrosis factor pathway

TNF is a potent pro-inflammatory cytokine which exerts diverse effects on a variety of cells and plays a critical role in the pathogenesis of chronic inflammatory diseases. TNF is produced primarily by activated macrophages, and also by monocytes, fibroblasts, B and T lymphocytes. (4,12) It is generated as a precursor form called transmembrane TNF (tmTNF) which is expressed on the surface of the cell after its synthesis. It is subsequently released as soluble TNF (sTNF) through the cleavage of membrane-anchoring domain by TNF-α-converting-enzyme (TACE). (4,12) Both types of TNF are homotrimeric which mediate their activities by interacting with Type 1 receptor (TNF-R1, also known as p55 receptor) or Type 2 receptor (TNF-R2, also known as p75 receptor) on the effector cells. (4,12,13)

The binding of TNF to its receptors triggers the recruitment of intracellular adaptor proteins, which leads to activation of complex intracellular signaling processes. Eventually, transcription factors such as nuclear factor kB (NF-kB) and c-Jun are activated and induce expression of genes important for immune and inflammatory response. (14-15)

TNF is a potent paracrine inducer of other inflammatory cytokines, including interleukin-1, interleukin-6, interleukin-8, vasodilatory PG12 and granulocyte-monocyte colony stimulating factors. TNF also promotes inflammation by stimulating fibroblasts to express adhesion molecules, which interact with their respective ligands on the surface of leukocytes, causing increase in transport of leukocytes into inflammatory sites. (4) Other inflammatory actions of TNF include stimulating B cell proliferation and antibody production, as well as enhancing natural killer cell cytotoxic activity. (4,12)
The level of circulating TNF in a healthy person is undetectable. However, TNF is found in high concentration in patients with inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases.\(^{(16)}\)

Anti-TNF agents currently available in Hong Kong

Anti-TNF agents have been successfully applied to the treatment of chronic inflammatory diseases with significant amount of data on the clinical profile. The three major types of anti-TNF agents registered and used in Hong Kong are infliximab (REMICADE\(^{®}\)), adalimumab (HUMIRA\(^{®}\)) and etanercept (ENBREL\(^{®}\)).\(^{(17)}\)

Structure and binding of anti-TNF agents

Infliximab and adalimumab are monoclonal antibodies (mAbs) against human TNF. Infliximab is a chimeric IgG1 antibody, while adalimumab is a recombinant human IgG1.\(^{(6-7)}\) Etanercept is a fusion protein made up of the extracellular portion of recombinant soluble p75 receptors (TNF-R2) linked to the Fc portion of human IgG.\(^{(4)}\) Originally, soluble receptors have short plasma half-lives so frequent dosing is required to effectively neutralize cytokines. Conjugating soluble receptors with the Fc portion can extend their half-lives to approximate that of human IgG.\(^{(4)}\)

Mechanisms of action and pharmacokinetics of anti-TNF agents

Action against soluble TNF

Anti-TNF mAbs (e.g. infliximab and adalimumab) and soluble TNF receptors (e.g. etanercept) prevent interactions of soluble or transmembrane TNF with cell surface TNF receptors, thus neutralizing their stimulating effects on effector cells.\(^{(5)}\) All three agents can neutralize human TNF, preventing it from binding to the cell-surface TNF receptor. They have similar intrinsic binding properties for sTNF in overall, but infliximab and adalimumab have greater degree of binding to tmTNF and form more stable complexes with tmTNF when compared to etanercept.\(^{(12-13)}\) Furthermore, these three agents have different TNF binding avidity, which is the combined synergistic strength of bond affinities. One infliximab or adalimumab molecule is able to bind up to 3 TNF molecules, and one TNF homotrimer can bind up to 3 molecules of anti-TNF mAb. However, etanercept binds to the junction of 2 TNF monomer units in a 1:1 ratio.\(^{(13,16)}\) Such binding differences are the possible reasons for different efficacy and side effect profile of the three agents.
**Action against transmembrane TNF**

In-vitro studies demonstrate that, when binding to tmTNF, anti-TNF agents can also destroy TNF-bearing cells by exhibiting complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and outside-to-inside signaling property.

**Outside-to-inside signaling**

Only infliximab and adalimumab, but not etanercept, possess this activity. The binding of infliximab and adalimumab to tmTNF triggers series of intracellular signaling events, inducing apoptosis and G0/G1 cell cycle arrest to the tmTNF bearing cells. (12)

It was found that anti-TNF mAbs can also inhibit the production of interferon-y (IFNy) apart from TNF. Infliximab and adalimumab significantly inhibit IFNy, but no significant inhibitory effect was observed with etanercept, (16) which is a possible reason for different safety profile of the three agents.

The serum half-lives of infliximab (9.5 days) and adalimumab (12-14 days) are much longer than that of etanercept (2.9 days). 6-7 2-4 Detectable level of free infliximab can be observed up to 28 weeks after a single dose. (16) The volume of distribution of anti-TNF mAbs are also larger than etanercept. Base on the pharmacokinetic differences, infliximab and adalimumab may have greater and more prolonged suppression of TNF than etanercept does.

**Indications of anti-TNF agents**

All three anti-TNF agents treats chronic inflammatory diseases but there are slight difference in their indications and usage.

**Infliximab**

Infliximab is administered by intravenous infusion. (7) It is indicated for moderately to severely active RA when used concurrently with methotrexate. The combination therapy demonstrates significant reduction in the signs and symptoms of RA, inhibition of the progression of joint structural damage and improvement in physical function in placebo-controlled studies. However, there is insufficient evidence to support the efficacy of infliximab monotherapy in the management of RA. (19)

Other indications of infliximab include for reducing signs and symptoms of Crohn’s disease and ulcerative colitis. It can also be used in the management of psoriatic arthritis, ankylosing spondylitis and chronic severe plaque psoriasis. (7)

**Adalimumab**

Adalimumab is administered by subcutaneous injection. (8) It can be used alone or in combination with DMARDs to treat RA. Several placebo-controlled clinical studies demonstrate that adalimumab are effective in the treatment of RA. Some clinical evaluations suggest that adalimumab-methotrexate combination therapy is more effective than therapy with either agent alone. (19) Beside, adalimumab is also indicated for moderately or severely active Crohn’s disease, psoriatic arthritis, ankylosing spondylitis (AS), plaque psoriasis or juvenile idiopathic arthritis (JIA) in patients age 4 years or above. (6)

**Etanercept**

Same as adalimumab, etanercept is administered by subcutaneous injection. (6) Etanercept is indicated for RA, psoriatic arthritis, AS, plaque psoriasis and JIA in patients of 2 years old or above. Clinical evaluations have shown that etanercept is more effective than placebo in the treatment of RA and is at least as effective as methotrexate for management of RA in adults. Some study results show that etanercept-methotrexate combination therapy is more effective than monotherapy of either agent alone. The addition of methotrexate to etanercept monotherapy for RA is optional. (19)

Unlike infliximab and adalimumab, etanercept is not effective in treating Crohn’s disease, which is a granulomatous disease. Granulomas are collections of macrophages and neutrophils.

<table>
<thead>
<tr>
<th>Table 1. Structure and Mechanism of actions of anti-TNF agents</th>
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<td><strong>Infliximab</strong></td>
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<td>CDC (in vitro)</td>
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<td>Outside-inside signaling (in vitro)</td>
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<td>Human fusion protein of IgG1 and p75 receptor</td>
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**Complement-dependent cytotoxicity**

Infliximab, adalimumab and etanercept possess the Fc portion of IgG1, which consists of CH2 and CH3 domains. CH2 domain activates complement C1 which simultaneously leads to the activation of complement C3 and subsequent formation of membrane attack complex (MAC, C5b-C9). The MAC causes lysis of tmTNF-bearing cells. Since etanercept does not possess CH1 domain of IgG1 for the activation of complement C3, it has much lower level of, or even no, CDC activity. (12)

**Antibody-dependent cell-mediated cytotoxicity**

Infliximab, adalimumab and etanercept showed similar ADCC activities in vitro. The CH2 and CH3 domains of the Fc portion involve in the binding to the Fc receptors of natural killer cells (NK cells). Granzyme B and perforin are released from NK cells and lyse tmTNF bearing cells. However, with the presence of soluble TNF in the assay system, only infliximab and adalimumab were found to have binding with Fc receptors on NK cells. (12)
multi-nucleated giant cells, which are encircled by lymphocytes. TNF, especially tmTNF, has been shown in recent years studies that it plays an important role in granulomas formation and the maintenance of granuloma architecture.\(^{(16)}\) Although all three agents have TNF antagonizing property, infliximab and adalimumab have stronger binding to tmTNF and result in macrophage and monocyte lysis by CDC, ADCC and outside-to-inside signaling. Anti-TNF mAbs may therefore have stronger anti-granuloma function than etanercept does.\(^{(12,16)}\)

**Safety concerns for anti-TNF agents**

Anti-TNF agents treat immunologic diseases by antagonizing the activity of TNF. Although they have more immune-specificity than conventional immunosuppressants, it has been reported that infections have been associated with the use of anti-TNF agents. Infliximab, adalimumab and etanercept all carry the safety warning of increased risk of serious infections, including tuberculosis (TB), bacterial sepsis, invasive fungal infections and infections due to other opportunistic pathogens. These infections may lead to hospitalization or even death. Active TB is the most frequently reported infection in patients treated with anti-TNF agents.\(^{(6,7-8)}\) Post-marketing surveillance in the US from 1998 through September 2002 identified that infliximab-treated patients have a 2- to 8-fold greater risk of granulomatous infection than that of etanercept-treated patients.\(^{(12,16)}\) In other European countries, it has been reported that greater incidence of TB have been observed in patients using infliximab and adalimumab, when compared to etanercept.\(^{(16)}\) The risk of granulomatous infections appears to be in relation to the high level of anti-granuloma activity of anti-TNF mAbs, just as what have been mentioned in *Indication*. Apart from that, etanercept binds TNF less avidly (the binding ratio of etanercept : TNF is 1:1) than the mAbs do, therefore etanercept may have less suppression on the immune resistance of human body. Longer serum half-lives and larger volume of distribution may also contribute to the higher risk of anti-TNF mAbs in causing serious infections.\(^{(16)}\) The routes of administration may affect the side effect profile of the agents as well. Etanercept and adalimumab are administered by subcutaneous injection, which gives a lower peak serum concentration and tissue concentration than that of infliximab, which is administered intravenously. Patient’s immunity is more likely to control granulomatous infections when drug concentration is lower.

Infliximab, adalimumab and etanercept carry a precaution warning about reported malignancies in patients who initiate their anti-TNF agents therapy at the age of ≤18 years. Lymphoma is the major type of malignancy reported.\(^{(6,7-8)}\) Post-marketing TNF-blocker use in RA and other indications have been reported to associate with leukemia. However, there are studies on RA patients find out that they may have a higher risk of developing leukemia than the general population even without the use of anti-TNF agents. Tumor necrosis factor was first identified for its ability to induce rapid haemorrhagic necrosis of experimental cancer.\(^{(21)}\) It is believed that TNF has anti-tumor activity and can be used as a treatment for cancer. Anti-TNF agents may precipitate malignancies as a result of blocking TNF anti-tumor property. However, the ultimate reason of increase malignancy risk is still unknown, especially the pro-tumor actions of TNF become apparent in recent studies.

Manufacturers recommend that immunization with live vaccines should not be given concurrently with infliximab, adalimumab or etanercept although there are no data on secondary infection by live vaccines or on the response after live vaccines administration in patients taking anti-TNF agents. Due to the concern of possible immunosuppression caused by anti-TNF agents, this precaution is to be exercised carefully by healthcare professionals. Patients can receive vaccinations, except for live vaccines.\(^{(6,7-8)}\)

Studies and post-marketing surveillance discover that there are possible association between the three anti-TNF agent with hepatitis B reactivation, lupus-like syndrome, hypersensitivity, heart failure, demylinating disease and immunosuppression. Precautions have to be taken before starting anti-TNF therapies. Common adverse reactions to the agents are infections, infusion-related reactions and injection site reactions.\(^{(5,7-8)}\)

**Immunogenicity of anti-TNF agents**

Although there are ample data supporting the clinical efficacy of anti-TNF agents in treating chronic inflammatory diseases, it is observed that some patients fail to respond to the agents, and some initial responders have diminished response upon prolonged anti-TNF treatment. One of the possible explanations could be the formation of antibodies against anti-TNF agents.\(^{(22-24)}\)

All three anti-TNF agents induce the formation of antibodies. As shown in the diagram (Fig. 6), the circled parts, even in 100% human structures, are the potentially immunogenic sites of the agents.\(^{(28)}\) Chimeric and human mAbs are expected to be less immunogenic than murine mAb so they are more suitable as therapeutic agents.\(^{(4)}\) However, epitopes on the variable region of infliximab still induces the production of Human Anti Chimeric Antibodies (HACAs), which has been observed in patients with RA,\(^{(22-23)}\) Adalimumab, although being a fully human structure, also induces Human Anti Human Antibodies (HAHAs) production in RA patients.\(^{(22,24)}\) The HACAs and HAHAs have neutralizing effect on the anti-TNF mAbs. They increase the clearance of anti-TNF mAbs and decrease their serum concentration, which is found to be associated with reduced response to the treatment and higher risk of development of infusion reactions. Concomitant use of immunosuppressive drugs or corticosteroids with anti-TNF mAbs have been proved to reduce the formation of neutralizing antibodies. Infliximab treatment requires concurrent

*Figure 6. Potential immunogenic sequence of anti-TNF agents.*\(^{(25)}\)
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. Why biologics are good alternatives to the existing therapeutic options of rheumatoid arthritis?
   I. Existing drug options are often inadequate to properly manage the disease state.
   II. Existing drug options are too expensive.
   III. The toxicities of existing drug options limit their use.
   A. I. only
   B. II. & III only
   C. I. & III only
   D. All of above.

2. Which of the followings are the pro-inflammatory actions of TNF?
   I. Inducing the production of other inflammatory mediators
   II. Stimulating B cell proliferation and antibody production
   III. Causing increase in transport of leukocytes into inflammatory sites
   A. I. only
   B. I. & III only
   C. II. & III only
   D. All of above.

3. Which of the following about infliximab is wrong?
   A. Infliximab is a chimeric monoclonal antibody.
   B. Infliximab blocks the interaction between cell-surface receptors and soluble TNF only.
   C. Infliximab can induce apoptosis of mTNF-bearing cells.
   D. There is insufficient evidence to support the efficacy of infliximab monotherapy in the management of RA.

4. Which of the following about adalimumab is wrong?
   A. Adalimumab has the longest half-life among 3 kinds of anti-TNF agents.
   B. Adalimumab is administered by subcutaneous injection.
   C. Adalimumab must be used with DMARDs to treat RA.
   D. Adalimumab induces Human Anti Human Antibodies (HAHAs) production.

5. Which of the following about etanercept is wrong?
   A. Etanercept has low level of complement dependent cytotoxicity.
   B. Etanercept possesses outside-to-inside signaling activities.
   C. Etanercept has smaller volume of distribution than anti-TNF monoclonal antibodies.
   D. Etanercept is not effective in treating Crohn’s disease.

6. Patients on anti-TNF agents can receive the following kinds of vaccinations except:
   A. Hepatitis A vaccine
   B. MMR vaccine
   C. Influenza vaccine
   D. Pneumococcal vaccine

7. HACAs and HAHAs:
   A. associate with higher risk of development of infusion reactions
   B. reduce the clearance of anti-TNF mAbs
   C. associate with increased response to the treatment
   D. formation cannot be reduced by concomitant use of immunosuppressive drugs

8. When compared to infliximab and adalimumab, etanercept
   A. is not immunogenic.
   B. produces a less stable plasma concentration between two injections.
   C. has no correlation between the formation of antibodies against etanercept, etanercept level and clinical response.
   D. is associated with greater incidence of TB.

9. Which of the followings are the possible side-effects of anti-TNF agents?
   A. Injection-site reaction or infusion-related reaction
   B. Increased risk of serious infection
   C. Hepatitis B reactivation
   A. I. only
   B. II. & III only
   C. I. & III only
   D. All of above.

10. Which of the following statement is correct?
    A. Any changes in manufacturing process, facilities or equipments could alter the biological molecules.
    B. Characterizing a biologic is easier than analyzing traditional chemically synthesized drug
    C. Molecular changes can be detected by standard characterization techniques
    D. Biological products do not meet the definition of drugs under the Federal Food, Drug and Cosmetic Act of the US.

Answers will be released in the next issue of HKPJ.
Absence of Chlorogenic Acid in Ginkgo biloba Leaf Samples and Their Over-the-Counter Products

ZHU, Lixia; GAO, Jingchun; BAIBADO, Joewel Tarra; CHENG, Yushan; CHAN, Kelvin K.C.; CHEUNG, Hon-Yeung

a Research Group for Bioactive Products, Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Hong Kong SAR, P.R. China.
b Centre for Complementary Medicine Research, University of Western Sydney, NSW 2560, and Faculty of Pharmacy, University of Sydney, NSW 2006, Australia

(*Corresponding author. Tel: +852 3442-7746; Fax :+852 3442 0522; Email: bhhonyun@cityu.edu.hk)

ABSTRACT

Ginkgo biloba leaves (also called Ginkgo Folia, GF) and their extracts are popular herbal products or nutritional supplements used worldwide. In both the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP), chlorogenic acid and rutin are suggested as reference markers for the TLC identification of Ginkgo leaves. We have found from both TLC assays and HPLC analysis that another compound which is present in Ginkgo Folia exhibits similar TLC behavior after heating to chlorogenic acid, and is in fact the real marker in TLC analysis. Further LC/MS/MS analysis of the compound, previously mistaken to be chlorogenic acid, revealed that it is 6-hydroxykynurenic acid, which is one of the many ingredients reported in Ginkgo leaves. Hence, we recommend that 6-hydroxykynurenic acid, instead of chlorogenic acid, should be used as the marker for quick TLC identification of the Ginkgo leaf and its derived functional food.

INTRODUCTION

The Ginkgo tree (Ginkgo biloba L.), also known as the Maidenhair Tree, is a living fossil because it is the sole surviving species of the family Ginkgoaceae. Ginkgo Folia (GF), which are the dried leaves of Ginkgo biloba L., have been employed as herbal medicine for centuries in China. These leaves have gained recognition for the improvement of blood circulation, both peripherally and centrally, and the positive effects upon dementia, memory, and cognition. Nowadays, Ginkgo biloba leaves have become one of the most widely used herbal products or dietary supplements around the world. The beneficial effects of GF are contributed mainly by two kinds of active ingredients: flavonoids (such as flavonol glycosides, isoflavonoids, biflavones and proanthocyanidins) and terpene lactones, such as ginkgolides A, B, C, J and bilobalide.

For quality control purposes, certain active components reported in Ginkgo biloba leaves have been recommended as chemical markers for comparison and identification of the herb when using thin-layer chromatography (TLC). In the Chinese Pharmacopoeia, ginkgolides A, B, C and bilobalide (Fig. 1) are used as reference standards for its TLC identification, while chlorogenic acid and rutin (Fig. 1) are suggested as reference markers for TLC tests of GF in both the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP). However, there has been no report demonstrating the occurrence of chlorogenic acid in GF so far, except for two studies conducted by the same researcher. The presence of...
chlorogenic acid in Ginkgo biloba leaves therefore remains in doubt and its use as a TLC marker is controversial. For this reason, a series of experiments based on TLC, HPLC and LC/MS/MS studies were carried out in our laboratory, with an aim to ascertain whether or not chlorogenic acid is present in Ginkgo biloba leaves, and to examine the use of this marker. It was found that chlorogenic acid is not present in Ginkgo biloba leaves or in their extracted commercial products.

EXPERIMENTAL

Reagents and Materials
Standard chlorogenic acid and rutin (98% purified) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Acetonitrile and formic acid used for HPLC were of chromatographic grade, and all other chemical reagents used were of analytical grade. The sample identifications and sources of GF samples collected from Mainland China (batches S1-S6) and Hong Kong (batches S7-S10) markets are listed in Table 1. A standard GF sample and extract were obtained from NICPBP (Beijing, China). A USP standardized GF extract was purchased from Hubei Ginkgo Product Co. Ltd. (Xuzhou, China), and a commercial GF extract was purchased from Vita Green Health Products Inc. (California, USA).

Apparatus
The TLC analysis was performed by using a semi-automatic TLC applicator (CAMAG Reprostar 3 lighting module and a digital camera (CAMAG, Muttenz, Switzerland). HPTLC plates pre-coated with silica gel 60 F254 (Merck Chemicals, Germany) were used. The HPLC analysis was performed on a Waters 2695 HPLC system coupled with a Waters 2996 detector. A GRACE Altima C18 column (250 mm×4.6 mm i.d., 5 μm) was used in the HPLC analysis. LC/MS/MS analysis was performed on an Agilent 1100 system (Palo Alto, California, USA) coupled with a Bruker Esquire-LC ion trap mass spectrometer (Bruker Daltonics, Massachusetts, USA). An Agilent Zorbax SB C18 column (250 mm×4.6 mm i.d., 5 μm) was used for HPLC–MS/MS analysis.

TLC Analysis
Ginkgo Folium samples were homogenized to fine powder, and 1.0 g of the powdered sample (or 0.2 g of the standard GF extract) was transferred into a 50-mL centrifuge tube. After addition of 10 mL of methanol, the sample mixture was sonicated (240 W) for 30 min. The mixtures were further filtered through a 0.45 μm pore size filter and the filtrate was used for TLC analysis after clean-up by pouring through a solid phase extraction column. Stock standard solutions of rutin (1500 mg/L) and chlorogenic acid (1000 mg/L) were prepared for TLC analysis carried out at 28ºC and 50% relative humidity. The TLC mobile phase systems employed, together with the spraying reagents used, are described in the legend of Figs. 2 and 3.

HPLC Analysis
In the HPLC analysis of the herb, 1.0 g of powdered GF sample was weighed into a 50-mL centrifuge tube. Then 20 mL of 60% methanol solution was added and the mixture was sonicated for 30 min and subsequently centrifuged at 4000 rpm for 10 min. The supernatant was filtered through a 0.45 μm pore size nitrocellulose membrane and the filtrate was used directly for HPLC analysis. A stock solution of 100 mg/L chlorogenic acid solution was accurately prepared for HPLC analysis and a series of solutions with the appropriate concentrations were prepared by dilution with methanol. The limit of detection (LOD) of chlorogenic acid was determined from the concentrations of serially diluted solutions until the S/N ratio was 3.

The mobile phase system was composed of (A) 0.1 v/v% formic acid aqueous solution and (B) 0.1 v/v% formic acid acetonitrile solution, with the gradient program as follows: 0–40 min, 10-26% (B); 40-60 min, 26-42% (B). The flow rate was 1.0 mL/min and the injection volume was 10 μL. Chromatograms were recorded at the detection wavelength of 326 nm.

Table 1. Source information of ten batches of Ginkgo Folium samples

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Sample ID</th>
<th>Source</th>
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<tbody>
<tr>
<td>S1</td>
<td>YXY-01-01</td>
<td>Yichang, Hubei</td>
</tr>
<tr>
<td>S2</td>
<td>YXY-02-01</td>
<td>Chizhou, Anhui</td>
</tr>
<tr>
<td>S3</td>
<td>YXY-03-01</td>
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<td>S4</td>
<td>YXY-04-01</td>
<td>Anguo, Hebei</td>
</tr>
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<td>S5</td>
<td>YXY-05-01</td>
<td>Unknown</td>
</tr>
<tr>
<td>S6</td>
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<td>Hunan</td>
</tr>
<tr>
<td>S7</td>
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<td>LXYX-09-01</td>
<td>Shanxi</td>
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<tr>
<td>S10</td>
<td>LXYX-10-01</td>
<td>Hubei</td>
</tr>
<tr>
<td>S11</td>
<td>Standard Ginkgo Folium</td>
<td>NICPBP</td>
</tr>
<tr>
<td>S12</td>
<td>OTC Ginkgo Folium</td>
<td>Vita Green Health Products Inc, USA</td>
</tr>
</tbody>
</table>

Figure 2. TLC results of Ginkgo biloba leaf extracts (GF) and chlorogenic acid under different conditions. Leaf extracts of GF sample were loaded onto a TLC plate and developed using the TLC method described in USP and BP. After developed, bands were visualized under 366 nm wavelength radiation. A = Freshly-run TLC plate before heating; B = The same plate examined after heating and spraying (lane 1 = Standard sample, 10 μL; 2 = S5, 10 μL; 3 = Chlorogenic acid, 4 μL); C = TLC results of GF samples from various sources examined under 366 nm wavelength radiation before heating and spraying; D = same as C but examined after heating and spraying (lane 1 = Blank, 10 μL; 2 = Rutin, 2 μL; 3 = Chlorogenic acid, 3 μL; 4, 5 = S5 sample in duplicate, 10 μL; 6 = S5 sample, 10 μL spiked with rutin, 2 μL and chlorogenic acid, 3 μL; 7 = Standard Ginkgo Folium from NICPBP; 8 = Standard Ginkgo Folium extract from NICPBP; 9 = Commercial Ginkgo Folium extract from Vita Green Health Products Inc.; USA; 10 = Chlorogenic acid, 3 μL.
LC-MS/MS analysis

The powder of the long pink band scraped from the TLC plate was transferred into a 1-mL centrifuge tube and mixed with 0.5 mL of methanol. The mixture was sonicated for 15 min and then centrifuged at 10000 rpm for 5 min. The supernatant was further filtered through a 0.45 μm pore size nitrocellulose membrane and used for LC-MS/MS analysis. The corresponding LC condition was that of the HPLC analysis described previously, except that the flow rate was 0.3 mL/min. The mass spectrometer was operated in the positive ion mode using an electrospray ionization source. The selected ion monitoring mode was operated at 206 m/z. The capillary exit voltage was 102.4 V and the pressure of nebulizer was 30.0 psi. The flow rate of the drying gas was 9.0 mL/min at the temperature of 280°C.

RESULTS AND DISCUSSION

TLC identification

The TLC results of the standard GF sample and chlorogenic acid, based on the developing solvent system recommended by both USP and BP, are displayed in Fig. 2. Fig. 2A depicts the scenario before heating the plate, and Fig. 2B after heating at 105°C for 3 min. As shown in Fig 2B, after the TLC plate was sprayed, a light blue fluorescent band appeared in the standard GF sample. This band exhibited a similar appearance and Rf value to that of the corresponding band of chlorogenic acid. However, when the unheated TLC plate was examined (Fig. 2A), a pink fluorescent band was observed in the GF sample, which was considerably different from the very light band of chlorogenic acid under the same conditions.

The GF samples from various sources were also analyzed by TLC. Most of the GF samples showed the pink fluorescent band before heating (Fig. 2C) and this changed to the light blue fluorescent band after heating and spraying (Fig. 2D). The same phenomenon was observed even when the sample volume was scaled down by a factor of two (data not shown).

Since a different image was observed for the GF samples and chlorogenic acid before and after heating, it is reasonable to doubt that the compound present in GF samples is chlorogenic acid.

Fig. 3 shows the result when another developing solvent system (n-butanol-pyridine-water, 14:3:3, v/v) was used in the TLC analysis of a GF sample and chlorogenic acid. The Rf value of the blue light fluorescent band observed in the GF sample, no matter whether before (Fig. 3A, lanes 1 & 2) or after heat treatment (Fig. 3B, lanes 1 & 2), is obviously different from that of chlorogenic acid (lane 4 of Fig 3A & B). This result again suggests that the blue light fluorescent band in the TLC of GF is not chlorogenic acid, and further supports our speculation that chlorogenic acid is not present in GF.

HPLC identification

For a further confirmation of the absence of chlorogenic acid in GF, HPLC analysis was conducted on different batches of GF samples. Fig. 4A shows chromatograms of chlorogenic acid, extract of GF sample (S5), and the S5 sample spiked with chlorogenic acid monitored at the wavelength of 326 nm. The LOD of chlorogenic acid was determined to be 0.1 mg/L. At about 12 min, which is the retention time of chlorogenic acid, no peak was observed for the GF sample (S5), indicating that chlorogenic acid is not present in S5 above our determined LOD. When other batches (S1 – S10) of GF samples were checked, chlorogenic acid was also not found (Fig. 5B). Therefore, the compound with blue light band recommended by USP and BP in the TLC analysis of GF, is not chlorogenic acid. Hence, it is confirmed that chlorogenic acid is not present in GF.

LC/MS/MS identification

In the following study, LC-DAD/MS/MS analysis was applied to identify the
that the nitrogen-containing phenolic acid 6-hydroxykynurenic acid with relative molecular mass 205 has been identified in GF samples. This acid is one of the major components in GF and its content was determined to be in the range from 0.121 - 0.269 mg/g in the commercial GF samples. Moreover, 6-hydroxykynurenic acid has been reported to have nearly the same performance and behavior as chlorogenic acid in TLC assays, including the light blue band and close *Rf* value. This compound shows a pink fluorescence under UV light and it is unstable under acidic conditions. Based on all our experimental data collected and upon these literature reports, it is concluded that the compound which has been mistaken for chlorogenic acid in GF is in fact 6-hydroxykynurenic acid (6-HKA, Fig. 5).

### CONCLUSIONS

Chlorogenic acid has been selected as a reference marker in the TLC identification of Ginkgo biloba leaves as recorded in both USP and BP. However, our findings indicate that chlorogenic acid is not present above our limit of detection in Ginkgo leaves, and that the compound which has a similar behavior to chlorogenic acid in TLC analysis under USP and BP conditions has been identified as 6-HKA. It has been reported that 6-HKA extracted from Ginkgo biloba leaves could inhibit glutamate receptors and that it exerts neuroprotective effects, which are also exhibited by other typical biological constituents of Ginkgo biloba such as bilobalides and ginkgobilalides. In addition, 6-HKA in Ginkgo biloba leaves has been shown to exhibit potent peroxynitrite scavenging activity which is believed to contribute to the reported actions of Ginkgo biloba. Therefore, it is suggested that 6-hydroxykynurenic acid instead of chlorogenic acid should be used as a marker in the TLC identification of Ginkgo leaves.

### ACKNOWLEDGMENTS

This study is a partial work of the project (CityU 9210029) of Hong Kong Chinese Materia Medica Standards (HKCMMs) funded by the Department of Health, Hong Kong SAR Government. The authors would also like to thank all members of the Scientific Committee and the International Advisory Board of the HKCMMs for their critical comments and advices throughout this study.

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**Author’s background**

**ZHU Lixia** is a registered pharmacist in China. She holds a MSc degree in Analytical Chemistry and a BSc degree in Chinese Medicine. She was a research associate at Shenzhen Applied Research Institute of City University of Hong Kong.

**Prof. BAIBADO Joewel T.** is a doctor of Public Health Medical Microbiology student at the University of the Philippines Manila. He is an assistant professor of Medical Microbiology & Human Biology at Illoilo Doctors’ College in Philippines. His research interests include screening of antimicrobial properties of bioactive compounds of Philippine mangroves and indigenous herbs. He is also a research associate at the City University of Hong Kong. He is responsible for the histologic preparation and microscopic identification of Traditional Chinese Medicinal herbs.

**Dr GAO Jingchun** holds a BSc and a PhD degree in Pharmacy. She was a Senior Research Fellow at Shenzhen Applied Research Institute of City University of Hong Kong. Dr **CHENG Yushan** is a Postdoctoral Fellow in City University of Hong Kong. He is a registered Pharmacist in China and has a PhD degree in Analytical Pharmacy.

**Professor CHAN Kelvin K.C.** is a veteran scholar in Pharmacology. He is a pharmacist and a well-known herbal expert in UK, Hong Kong and Australia. He is now a joint Professor of Alternative Medicines in University of Sydney and University of Western Sydney. He is also a Scientific Committee member and an International Advisory Board member of the HKCMMs for the Department of Health, Hong Kong SAR Government.

**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 35 years of work experience in industry, academic and consultancy jobs. He has been an expert witness in court and a member of the Biotechnology Committee for Hong Kong and Shenzhen Government. Dr. Cheung has published more than 200 papers and articles in many prestigious international journals. His email address: bhonyun@cityu.edu.hk
ABSTRACT

A method for preparative separation, characterization and determination of rutin, isorhamnetin-3-O-[6’’-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol from *G. pentaphyllum* was successfully established in this paper. The extracts, using 50% ethanol as extractant, were then purified by elution extrusion counter-current chromatography (EECCC) with n-hexane/ethyl acetate/methanol/water (5:6:5:6, v/v/v/v) as the first dimension and ethyl acetate/n-butanol/water (4:1:5, v/v/v) as the second dimension. The compounds obtained can be used as reference substances for chromatographic purposes and each compound was characterized and determined by information-dependent acquisition mediated ultra-performance liquid chromatography tandem mass spectrometry (IDA−UPLC−MS/MS).

INTRODUCTION

*Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae), a perennial creeping herb called *jiao-gu-lan* in Chinese, is widely distributed in China, Japan, Korea and Southeast Asia in warm and humid environments (Figure 1). Its aerial part is used as a folk medicine to alleviate various diseases and symptoms including hypertension, cough, migraine, insomnia and diabetes mellitus.(1) Recently, much attention has received from scientists throughout the world in extensive phytochemical and pharmacological studies on this species, because of the unique structures and various biological activities of the relative compounds. A broad spectrum of beneficial effects had been reported, including antioxidative activity,(2) anti-inflammation,(3) anti-hyperlipidemia,(4) antitumour(5) and anti-cancer effect,(6) and has been described as having minimal toxicity. Main components of extracts from *G. pentaphyllum* are more than 100 dammarane-type triterpenoid saponins, several amino acids and vitamins, and trace elements.(7) Because of the similarity in bioactive components to the expensive *Panax ginseng* (Araliaceae), cheap *G. pentaphyllum* earned its favorable name of “Southern Ginseng”.(8) Therefore, the cultures of *G. pentaphyllum* or their extracts for health care have been put into production on a large scale.(9)

Figure 1. Picture of fresh *G. pentaphyllum* leaves (left), and modern tea beverage *G. pentaphyllum* (right).
HSCCC, developed by Ito in the late 1960s, is a liquid-liquid partition chromatography process which relies on the partition of a sample between two immiscible solvents to achieve separation. HSCCC is a preparative separation technique with high recovery, acceptable efficiency and without any solid matrix. In 2003, Berthod et al. proposed an elution-extrusion counter-current chromatography (EECCC), which extends the hydrophobicity window of the CCC technique and extrudes the most retained solutes out of the column with satisfactory peak resolution. Due to the fact that band broadening inside chromatographic columns depends only on the band position, the retained solutes in the stationary phase can be eluted out in the extrusion stage with relative good resolution, which saves a lot of time and solvent compared to standard CCC method. Due to the distinctive features, CCC has obviously played an important role in analysis and separation of various traditional Chinese herbs and other natural products.

Recently, several analytical methods have been developed for the analysis and determination of bioactive chemicals in herbal medicines. High-performance liquid chromatography (HPLC) method has been frequently used because of its high sensitivity and broad linear range. HPLC coupled with mass spectrometry (MS) has been favored by many analysts due to their higher sensitivity and their ability to provide compound confirmation. However, single MS spectra are produced that are not resolved from background ions, with overlapping chromatographic peaks and poor interlaboratory reproducibility. Tandem mass spectrometry produces very clean spectra, devoid of contaminant ions and thus easily amenable to library searching. However, it requires beforehand knowledge of the expected compounds in order to apply classic multiple-reaction monitoring (MRM) or product ion scan methods, which is not compatible with the objective of a general unknown screening technique. IDA is an artificial intelligence-based product ion scan mode, in which peaks found by SRM (survey scan) will instantly trigger the product ion scan (dependent acquisition) of the precursor ion of the eluting analyte. In this way, the high sensitivity of the SRM analysis is merged with the specificity of a product ion spectrum in a single analytical run.

In the present paper, an efficient method for the preparative isolation and purification of the major compounds from crude extract of G. pentaphyllum was established by CCC. Characterization and determination of the four individual chemicals, including rutin, isorhamnetin-3-O-[6"-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol were accomplished by use of IDA mediated UPLC coupled with electrospray ionisation mass spectrometry (IDA–UPLC–ESI-MS/MS). To the best of our knowledge, the preparative separation, characterization and determination of major compounds in G. pentaphyllum is now reported for the first time.

MATERIALS AND METHODS

Materials

The compounds studied for optimization of mass spectrometric parameters, LOD determinations and quantitative evaluation were rutin, isorhamnetin-3-O-[6"-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol (Figure 2). All solutions prepared for analysis were Milli-Q system (Millipore, Bedford, USA). Acetonitrile (ACN) and methanol (MeOH) were chromatographic grade purchased from Merck, Co. Inc. (Darmstadt, Germany). Formic acid (purity 96%, HPLC-grade) was provided by Tedia Company Inc. (Fairfield, OH, USA). All other chemicals were in analytical grade or better. High purity water with a resistivity of 18.2 MΩ cm⁻¹ was purified using a Milli-Q system (Millipore, Bedford, USA). All solutions prepared for analysis were passed through a membrane filter (0.22 μm pore size) before use.

Instrumentation

CCC separations were performed on a semi-preparative apparatus with one 150 mL coil and a counterweight, with multilayer coil prepared by winding a 48 m x 2 mm i.d. PTFE tube. The revolution speed of the apparatus can be regulated with a speed controller in the range of 0 and 1000 rpm and the sample injection was accomplished by an injection valve with a 2-mL sample loop. Furthermore, a Waters 515 flow pump (Waters, Milford, MA, USA) was used to fill the CCC apparatus with the stationary phase and to elute the mobile phase. The effluent was continuously monitored at 254 nm. Eluents was collected by an auto collector.

![Figure 2. Chemical structures of the major compounds in crude extract of G. pentaphyllum including rutin, isorhamnetin-3-O-[6"-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol.](image-url)
Chromatographic analysis was performed on a Waters Acquity UPLC system (Waters, Milford, MA, USA) which was equipped with a quaternary pump, an autosampler, a vacuum degasser and a LC workstation. The analytes separation was achieved on a Halo fused-core C18 column (50 mm × 2.1 mm, 2.7 μm particle size; Advanced Materials Technology, USA).

A triple-quadrupole linear ion trap mass spectrometer (4000 Q-trap, Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray™interface was used. Ionization was achieved using electrospray (ESI) source operating in the negative mode [M−H]− and the data were collected in the MRM mode. Instrument control, data acquisition and the processing were performed using the associate Analyst 1.5.1 software.

Sample preparation

The dried samples of G. pentaphyllum were bought from Jinkang Herbal Medicines Co. Ltd. (Nanshan, Hunan, China). Leaves were collected and powdered to a homogeneous size by a grinder, sieved through a No.40 mesh sieve (pore size 450 μm). A mass of 10 g pulverized powder was accurately weighted in to a 200 ml centrifuge bottle and mixed with 100 ml 50% aqueous ethanol. The mixture was placed on a thermostatic water bath and incubated at 50°C for 4h. After cooling down to room temperature, the bottle was subsequently centrifuged for 5 min at 6000 rpm. The supernatant solution was transferred and the dregs were re-extracted. Then, the extractions were combined and the resulting solution was filtered through filter paper. A portion of 1.0 mL filtrate were accurately measured, diluted 10-fold and filtered through a 0.22 μm filter before being analyzed by IDA-UPLC-MS/MS. As for the recovery test, three different concentration levels (high, middle and low) of the standard solutions were added to the powders of G. pentaphyllum prior to extraction. The resulting samples were extracted and analyzed as described above. Triplicate experiments were performed at each level.

CCC and UPLC–MS/MS conditions

The solvent system, containing the chosen ratios of n-hexane/ethyl acetate/methanol/water (5:6:5:6, v/v/v/v) as the first dimension and ethyl acetate/n-butanol/water (4:1:5, v/v/v) as the second dimension, was prepared, equilibrated and separated shortly before CCC separation. About 0.2 g crude sample was dissolved in a solvent mixture consisting of equal volumes of upper and lower phases. In CCC preparative separation run, the CCC column is first filled with the upper organic phase as the stationary phase. Then the coils are rotated at 800 rpm speed and the lower aqueous phase (the mobile phase) is pumped at the selected flow rate in the head-to-tail direction (reversed mode). When hydrodynamic equilibrium is established indicated by that the mobile phase exits the column outlet instead of the displaced stationary phase, the sample solution could be injected. The effluent is continuously monitored by UV and automatically collected in test tube per 5 min using the fraction collector. The chromatogram is first developed in a classical way. For EECCC separation, the stationary phase (instead of the mobile phase) is pumped into the column without changing the flow rate, flow direction or rotor rotation speed. This phase change marks the beginning of the extrusion step. The fraction label as yellow color was evaporated to dryness and re-subjected to HSCCC separation as the second dimension.

The mobile phase consisted of (A) high purity water (0.1% formic acid) and (B) acetonitrile. A gradient elution program was applied as follows: 0–2.5 min linear increased from 5 to 20% B, 2.5–8 min linear increased from 20 to 50% B. The flow rate was 0.4 mL·min⁻¹.

All of the target compounds were eluted within 8 min. During the rest time the column was cleaned, readjusted to the initial conditions and equilibrated. The column temperature was set at 30°C, and injection volume of 2.0 μL was selected.

The QTrap mass instrument was operated in negative ionization mode. In order to increasing sensitivity, the ion source temperature (TEM) was set at 450°C, and ion spray voltage (IS) was always set at −4.5 kV. Ion source gas1 (GS1) and ion source gas2 (GS2) were used as the drying and nebulizer gases at a back pressure of 25 psi and 30 psi, respectively. Curtain gas (CUR) was set at 15 psi.

IDA mode was used to acquire the MS/MS spectra of the major compounds in G. pentaphyllum samples. The UPLC and MS parameters used in the IDA experiments were identical to that used in the LC–MS experiment. Using the IDA functionality in Analyst 1.5.1, three experiments were combined in each scan period within 1.2 s, one enhanced MS survey (EMS) scan and two MS/MS enhanced product ion (EPI) scans with different collision energies (CE). For the product ion scans, the resolution of the mass resolving quadrupole (Q1) was set low in order to speed up the scan rate (4000 Da/s), and the CE used were 20 and 40 eV, respectively. The ion peaks with peak intensity exceeding 1000 counts in the survey scan triggered MS/MS analyses. Former target ions that had been selected for MS/MS analyses were excluded from MS/MS analyses for 30 s.

RESULTS AND DISCUSSION

EECCC

The 50% ethanol extract of G. pentaphyllum provided a mixture of compounds with a wide range of polarities. Rutin, isorhamnetin-3-O-[6"-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol which were primarily identified by LC–MS/MS, were the most abundant and corresponded to peaks S1, S2, S3 and S4, in the HPLC chromatogram (Figure 3), respectively. Appropriate solvent system plays an important role in separation by CCC. Partition coefficient (K) is the most important parameter in solvent system selection, which should be close to 1 to get an efficient separation and a suitable run time. If it is much smaller than 1, the solutes will be eluted close to each other near the solvent front, which may result in loss of peak resolution; if the K value is much greater than 1, the solutes will be eluted in excessively broad peaks, and may lead to extended elution time.(23) In the preliminary experiment, various solvent systems based on n-hexane, ethyl acetate, methanol and water was conducted partition coefficient tested. Among the solvent systems, n-hexane/ethyl acetate/methanol/water (5:6:5:6, v/v/v/v) gave suitable partition coefficients for isorhamnetin-3-O-
[6'-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol. While the K value for rutin was somewhat low, leading to the co-elution with the polar matrix. Therefore, another dimension was utilized with solvent system ethyl acetate/n-butanol/water (4:1:5, v/v/v). Eventually, the major compounds from the crude extracts of G. pentaphyllum were well separated with high purities using offline two-dimension CCC as shown in Figure 4.

**UPLC–MS/MS conditions**

An efficient LC separation is important to avoid or reduce matrix effects. In addition, the selection of the flow rate can be relevant to the separation efficiency. Therefore, a serial of preliminary experiments were performed, testing different mobile phases flow rate in the range from 0.1 to 0.5 mL-min⁻¹. Low flow rate (≤ 0.2 mL-min⁻¹) usually makes total analysis procedure time consuming. However, when the flow rate reached the point at 0.5 mL-min⁻¹, the backpressure of the UPLC column was nearly to 10,000 psi, making the PEEK tube prone to breakup. Finally, the flow rate of 0.4 mL-min⁻¹ was selected, since the system gave the best signal/noise ratio, satisfactory efficiency and better peak profile.

Electrospray ionization (ESI) was tested in both positive- and negative-ion modes. rutin, isorhamnetin-3-O-[6'-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol showed much higher response signals using negative mode ESI than in the positive mode. Therefore, the ESI source in negative mode was chosen for target analytes detection. In order to quantitatively study the rutin, isorhamnetin-3-O-[6'-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol in crude extract of G. pentaphyllum, full scan spectra and MS/MS spectra were optimized to obtain the maximum response of signal for each compound by direct continuous pump infusion of standard working solutions of the standard solutions (100 ng·mL⁻¹) individually at a flow rate of 10 μL·mL⁻¹ in the mass spectrometer as shown in Figure 6. Identification of the precursor ions and optimum ionization conditions were performed in the full scan spectra and the product ion spectra was monitored to obtain the optimum collision energies (CE) as shown in

![Figure 4. EECCC spectrum of crude extract from herbal medicine G. pentaphyllum. Figure (A) is the first dimension, while Figure (B) is the second dimension.](image)

![Figure 5. The software interface showing a representative result of IDA–UPLC–MS/MS.](image)

![Figure 6. Total ion current of the EMS spectra.](image)
Figure 7. Product ion spectrum of the four preoperatively separated compounds.

The IDA used in this study allowed the mass spectrometer to collect the data of MS and MS² of the most intense ion at each time point from the UPLC running. In each IDA process, large amount of MS2 spectra from the EMS full scan were obtained. By exploring such a great deal of data and referring related references, the strategy used for structural characterization of major compounds in G. pentaphyllum could be facely conducted. Figure 5 showed the software interface and one representative result of IDA spectrum. However, one drawback of an IDA procedure might be due to the impossibility of optimizing the fragmentation parameters for each different precursor during the product ion scan. The use of a CE common to all compounds might preclude all chance of reaching a good fragmentation, especially if very different chemical structures are involved. (23)

Quantitative performance

Parameters of performance of the described IDA–UPLC–MS/MS method were determined and evaluated according to the considerations proposed in previous studies using spiked samples at levels of 0.1, 1.0 and 10.0 ng·mL⁻¹. (20) The values of the linearity, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ), recovery and stability were determined.

Linearity

The linearity of the response was determined by using a linear regression model. The matrix-matched calibration curves were analyzed with six levels of concentration, using the peak area of analyte versus the concentration of analyte with a weighting factor of 1/x. Each point of the curves had been injected at least in triplicate. The linear equations, correlation coefficients and linear range of the drugs are presented in Table 1. Each calibration curve was

Table 1. Parameters of regression equation, linearity range, limit of detection and quantification for Rutin, isorhamnetin-3-O-[6”-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Calibration curves</th>
<th>R²</th>
<th>Linear range (ng·mL⁻¹)</th>
<th>LOQ (ng·mL⁻¹)</th>
<th>LOD (ng·mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>y = 0.128x – 0.0513</td>
<td>0.9978</td>
<td>~5.0</td>
<td>0.020</td>
<td>0.064</td>
</tr>
<tr>
<td>isorhamnetin-3-O-[6”-rhamnosyl(1→6)]</td>
<td>y = 0.142x + 0.0252</td>
<td>0.9964</td>
<td>~5.0</td>
<td>0.010</td>
<td>0.301</td>
</tr>
<tr>
<td>glucopyranoside</td>
<td>y = 0.124x – 0.0127</td>
<td>0.9994</td>
<td>~5.0</td>
<td>0.028</td>
<td>0.084</td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>y = 0.112x + 0.0121</td>
<td>0.9985</td>
<td>~5.0</td>
<td>0.032</td>
<td>0.068</td>
</tr>
<tr>
<td>cirsiliol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calibration curves were constructed between the peak area ratio (y) and investigated compound concentration (x, ng·mL⁻¹).
* Linearity was studied ranging from LOQ value.
* Determined at S/N = 10.
* Determined at S/N = 3.

Table 2. The method precision at three different concentrations for online SPE–UPLC–MS/MS of Rutin, isorhamnetin-3-O-[6”-rhamnosyl(1→6)] glucopyranoside, isorhamnetin and cirsiliol from crude extracts of G. pentaphyllum samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Spiked levels (ng·mL⁻¹)</th>
<th>Recovery</th>
<th>Intra-day precision (n=7)</th>
<th>Inter-day precision (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value (ng·mL⁻¹) ± SD</td>
<td>RSD %</td>
<td>RSD %</td>
<td></td>
</tr>
<tr>
<td>Rutin</td>
<td>0.1</td>
<td>87.54</td>
<td>0.88 ± 0.03</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>92.38</td>
<td>1.05 ± 0.04</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>94.22</td>
<td>9.68 ± 0.58</td>
<td>3.20</td>
</tr>
<tr>
<td>isorhamnetin-3-O-[6”-rhamnosyl(1→6)]</td>
<td>0.1</td>
<td>92.06</td>
<td>1.07 ± 0.08</td>
<td>2.54</td>
</tr>
<tr>
<td>glucopyranoside</td>
<td>1.0</td>
<td>91.69</td>
<td>1.03 ± 0.05</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>94.37</td>
<td>10.06 ± 0.24</td>
<td>1.97</td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>0.1</td>
<td>88.85</td>
<td>1.02 ± 0.04</td>
<td>3.56</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>89.37</td>
<td>1.18 ± 0.54</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>81.09</td>
<td>11.60 ± 0.79</td>
<td>5.64</td>
</tr>
<tr>
<td>cirsiliol</td>
<td>0.1</td>
<td>92.36</td>
<td>0.97 ± 0.04</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>89.68</td>
<td>1.05 ± 0.05</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>91.48</td>
<td>9.89 ± 0.05</td>
<td>2.51</td>
</tr>
</tbody>
</table>
linear in a concentration ranging from the quantification limit to 50 ng·mL⁻¹ for each analyte, with satisfactory average correlation coefficients (0.9964–0.9994), which indicated good linearity between the peak area ratio (y) and investigated compound concentration (x, ng·mL⁻¹).

Accuracy and precision

Precision of the method was evaluated as intra- and inter-day precision by measuring corresponding relative standard deviations (RSDs) at three fortified concentrations (0.1, 1.0 and 10.0 ng·mL⁻¹) on three sequential runs in six replicates. Intra-day precision (repeatability) were measured on a single day using three replicates of each spiked matrices under the same conditions (same analyst, apparatus, reagents and short interval of time) where as inter-day precision was calculated during four consequent days using also three replicates of each matrices spiked at same concentration. The intra-day repeatability evaluated as relative standard deviation (RSD) ranged from 1.52 to 5.64% and for the inter-day repeatability was lower than 6.85% for all analytes (Table 2). All results were within the acceptable range and did not exceed 15% therefore we concluded that the method is accurate and precise.

LOD and LOQ

The most accepted definition of LOD and LOQ were considered as the analyte minimum concentrations that can be confidently identified and quantified by the method, respectively. The results of LOD and LOQ for rutin, isorhamnetin-3-O-β-D-glucopyranoside, isorhamnetin and cirsiliol were obtained from the extract of G. pentaphyllum by EECCh successfully for the first time. The compounds obtained can be used as reference substances for chromatographic purposes and each compound was characterized and determined by IDA–UPLC–MS/MS. The results of the present study clearly demonstrated that HSCCC coupling with IDA–UPLC–MS/MS is an efficient way for separation, characterization and determination of major compounds from natural sources.

CONCLUSION

Followed by suitable two-dimension solvent system, rutin, isorhamnetin-3-O-β-D-glucopyranoside, isorhamnetin and cirsiliol were obtained from the extract of G. pentaphyllum by EECCh successfully for the first time. The compounds obtained can be used as reference substances for chromatographic purposes and each compound was characterized and determined by IDA–UPLC–MS/MS. The results of the present study clearly demonstrated that HSCCC coupling with IDA–UPLC–MS/MS is an efficient way for separation, characterization and determination of major compounds from natural sources.

Author’s background

WANG, Yizuan is a visiting research assistant in the Shenzhen Applied Research Institute of the City University of Hong Kong. She is a full-time staff in the Department of Food Science and Technology, Zhejiang Gongshang University, Hangzhou. 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 30 years of work experiences in industries, academic and consultancy jobs. He was an expert witness in court and a member of the Biotechnology Committee for Hong Kong and Shenzhen Government. Dr. Cheung has published more than 200 papers and articles in many prestigious international journals. His email address: bhhonyun@cityu.edu.hk

References

Bioactive Substances and Medicinal Effects of Lycii Cortex

ZHANG, Zhi-Feng; LI, Yuan-Yuan; BAIBADO, Joewel Tarra; CHEUNG, Hon-Yeung*
Research Group for Bioactive Products, Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, China (*Corresponding author. Tel.: +852 3442 7746; Fax: +852 3442 0522; E-mail address: bhhonyun@cityu.edu.hk.)

Botanical Name: Lycium chinense Mill.; Lycium barbarum L.
Family: Solanaceae
Common names/other names: Qi gen, Di jie, Hong yue fu gen, Gou nai zi gun
Chinese Name: 地骨皮 (Digupi)
Part Usually Used: Root bark
Common Uses: Clearing heat and cooling blood

ABSTRACT

Lycium barbarum and L. chinense are two important species of the genus Lycium (Solanaceae) that have been used as herbal medicine. The root bark of these herbs is recorded as Lycii Cortex in the Pharmacopoeia of the People’s Republic of China. It is frequently used in clearing heat and cooling blood agent. A great number of secondary compounds including alkaloids, amides, peptides, flavonoids, coumarins, lignans, terpenoids, organic acids, sterols and steroids have been isolated and structurally identified from the root bark of Lycium chinense Mill. and Lycium barbarum L. Modern pharmacological studies revealed that extracts or some compounds isolated from Lycii cortex have many biological activities including anti-hypertensive, anti-diabetic, anti-inflammatory, antioxidant, antibiotic, and anti-parasitic. Hence, it is worthwhile to develop and explore this Chinese Materia Medica.

Keywords: Lycii Cortex, Microscopy identification, Chemical compounds, Bioactivity

INTRODUCTION

All over the world, the genus Lycium (family Solanaceae) comprises about 80 species. In China, there are seven species and three varieties are heavily distributed in the northwest and northern China. Lycium chinense Mill. and Lycium barbarum L. are the two important species in this genus. They are indigenous to Ningxia, Xinjiang and Hebei Province, China. The root bark, known as “Digupi” in Chinese, was firstly documented in “Shen Nong Ben Cao Jing” (神農本草經) and has been recorded as Lycii Cortex in the Pharmacopoeia of the People’s Republic of China. It is frequently prescribed as a component in traditional Chinese medicine for clearing heat and cooling blood. It is also applied to lower consumptive fever due to Yin deficiency. A decoction of the root bark is effective for the treatment of pneumonia, inflammation, night-sweats, cough, hematemesis, cough, and diabetes mellitus.

DESCRIPTION AND IDENTIFICATION

Macroscopic appearance

Lycii Cortex is the dried root bark of Lycium chinense Mill. or Lycium barbarum L (Fig.1). The root is collected in early spring and late autumn, removed from soil, washed, stripped off the root bark, then dried under the sun immediately to obtain it. It has been found that there is no significant difference in the morphological features between the two species except their size. In general, the root bark of the former is bigger and thicker than the latter. The root barks are quilled, semi-quilled or irregular, 3-12 cm long, 5-30 mm in diameter, 1-5 mm thick. Outer surface is greyish-yellow to brownish-yellow, coarse and loose, with longitudinally fissure, and the cork easily exfoliated. Inner surface is yellowish-white to greyish-yellow, relatively even, with fine longitudinal wrinkles. The texture is light and fragile, easily broken, fracture uneven or somewhat granular. It has a slight odour, and a sweet and sometimes bitter taste.

Microscopic appearance

There is no significant difference in the transverse section of the two species.

Figure 1. Pictures of a Lycium Chinense (A) and Lycium barbarum (B) (1. Flower 2. Fruits 3. Root barks)
The rhytidome is relatively thick. The cork is composed of 4-10 or even more layers of cells which are thin, flat, and lengthened tangentially. The cortex is narrow and is made up of cells that are elongated and surrounded with clefts. It has a broad phloem. The phloem rays are usually made up of 1 layer of cells. The fibres are occasionally visible and mostly scattered singly with thickened lignified walls. The parenchymatous cells contain abundant sand crystals of calcium oxalate (Fig. 2).

Cork cells are pale brown, polygonal or square (Fig. 3).

**BIOACTIVE COMPOUNDS**

Secondary metabolites have been isolated and structurally determined from the root bark of L. barbarum and L. Chinese. According to their chemical properties, these compounds are either belong to alkaloids, amides, peptides, flavonoids, coumarins, lignans, terpenoids, organic acids, sterols or steroids.

**Alkaloids**

Different kinds of alkaloids have been found and identified from the root barks. The structures of these alkaloids are shown in Fig. 4. Nortropane alkaloids which are comprised of 14 calystegines (1-14) are mainly present in the root bark of L. chinese.\(^7\) Two polyhydroxylated piperidine alkaloids (15-16) and its analogue (17) have also been identified from root bark of L. Chinese.\(^8\) Besides, three tropane alkaloids named atropine (18), scopolamine (19) and hyoscyamine (20) were identified in plants of L. barbarum\(^6\) and L. halimifolium.\(^8\) It was reported that L. halimifolium collected in India contained high contents of these toxic constituents.\(^6\)

More researchers believed and confirmed that the toxic tropane alkaloids are in trace amount in the fruits of Lycium genus. This means that the berries of Lycium can be safely used as food or medicine. However, the contents of the tropane alkaloids in the root or the root bark have not been investigated in details. In addition, two pyrrolidine alkaloids (21-22) namely, kukoaamine A (23) and kukoaamine B (24) plus the two spermine alkaloids have been isolated from root bark of L. Chinese.\(^5\) Two common alkaloids, betaine (25), choline (26), and indole glycoside (27) were also isolated from the root bark of Lycium genus.\(^5\)

**Amides**

Four phenolic amides (28-31) and Lyciumamide (32) were isolated from root barks of L. barbarum in 1987.\(^13,15\)

**Peptides**

Four octapeptides namely, Lyciumin A, B, C, and D (33-36) were identified from root barks L. chinese and L. barbarum.\(^16,17\)

**Flavonoids**

About twenty flavonoids were identified in Lycium genus.\(^12,18\) Most of them were found in the leaves, fruits, and flowers. Only apigenin (37), acacetin (38), luteolin (39), kaempferol (40), quercetin (41) and linaarin (42) were found in the in root barks (Fig. 5).\(^19-20\)
Anthraquinones

Two literatures published in Chinese revealed that series of anthraquinones (43-46) were existed in the root barks (Fig. 5).19, 21

Lignanoids

One lignanoid, (+)-lyoniresinol 3α-O-β-D-glucopyranoside (47) was isolated from the root bark of L. Chinese (Fig. 5).33

Coumarins

Coumarins, including scopoletin (48), scopolin (49), and fabiatrin (50) were identified in the root barks (Fig. 5).19

Organic acids

Night aromatic acids and derivatives were found in Lycium genus.12, 18 So far, only cinnamic acid (51), vanillic acid (52), syringic acid-O-glucoside (53), digupigan A (54) and p-coumaric acid (55) have been discovered in root barks.14, 19, 22 Besides aromatic acids, two fatty acids α-dimorphhecolic acid (56) and 9-hydroxy-E-10, Z-12, Z-15 octadecatrienoic acid (57) were also isolated (Fig. 6).15

Other compounds

There were still some terpenoids, sterol and derivatives isolated, including lyciumoside I~III, sugiol, 5α-stigmastane-3, 6-dione, β-sitosterol β-D-glucopyranoside, and furostanol glycoside (Fig. 6).16, 22, 23

Figure 4. Structures of some alkaloids, amides, and peptides isolated from L. chinense

Figure 5. Structures of flavonoids, anthraquinones, coumarins and lignans identified from L. chinense and L. barbarum.
QUALITATIVE AND QUANTITATIVE DETERMINATION OF BIOACTIVE COMPONENTS IN LYCII CORTEX

Although a lot of secondary compounds have been isolated from Lycii Cortex, less attention was paid to the quality control research with regards to bioactive constituents. Until now, the quality criteria recorded in the newest edition of Chinese Pharmacopoeia (2010 edition) is only requiring the content analysis of total ash, water, acid-insoluble ash and hot ethanol extract. None of the representative compounds was selected as the marker for identification or assay with the specific chromatographic method (i.e., HPLC and TLC). There are some literatures about determination of bioactive compounds in Lycii Cortex, and the results are summarized in Table 1. The markers selected were always lack of specificity (i.e., aromatic acids, flavonoids and coumarins) which were widely distributed in different kinds of medicinal plants. The contents determined vary significantly among different papers. Until now, it is still very difficult to judge whether such significant variations in the determination of the results were coming from the discrepancy of sample or from the system error of method adapted.

BIOLOGICAL EFFECTS

Lycii Cortex is a famous cooling agent with long history in TCM. Modern pharmacological studies revealed that extract or some isolated compounds have many biological effects. The following is the summarized bioactivities of Lycii Cortex in eight aspects. Most of the pharmacological investigation focused on L. chinese, whereas, some of the researches did not refer to the specific species.

Anti-diabetic effects

Chan et al. (2008) investigated the anti-diabetic activity of herbal formula SR 10 which contained Cortex Lycii as a key ingredient. This formula was determined to be effective in decreasing the blood glucose level for diabetic +db/+db mouse model. The reactive oxygen species (ROS) scavenging activity was considered as a contributor for protection of β-cell damage/apoptosis which was related to the development of type 2 DM. (20) Furthermore, the anti-diabetic effect of SR 10 was determined on streptozotocin treated pancreatic β-cells in vitro. It was found that SR 10 can reduce apoptosis β-cells by decreasing DNA fragmentation, sub-G1 peak area and percentage of apoptotic cells. Nitric oxide (NO) production in streptozotocin treated cells was inhibited though suppression of the expression of inducible nitric oxide synthesis (iNOS). (20) This research group further confirmed the inhibitory effect of SR10 on low-density lipoprotein oxidation, and vascular smooth muscle cell proliferation and migration. (21)

The effects of Lycii cortex ethanol and aqueous extract were evaluated on insulin resistance and lipid metabolism in obese-diabetic rats. The results suggested that rats administrated with both extract had decreased body weights, concentration of serum glucose, triglyceride (TG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), while significantly increased the insulin-sensitivity index (ISI). Ethanol extract seemed more effective than aqueous extract in these functions. However, constituents which contributed to these activities were not discussed in detail. (22)

The administration of Lycii cortex extractive can alleviate the increase in the degree of blood glucose and lipid in diabetic mice through improving abnormal glucose metabolism and insulin secretion. This function was considered to be related with restoration of impaired pancreas β-cell in alloxan-induced model. They further indicated that the effective chemical constituents were supposed to be organic acid, flavone, alkaloid, polysaccharide, anthraquinones and saponin through thin-layer chromatography (TLC). (20-24)

Asano et al. (1997) discovered 14 calystegines and several related alkaloids from L. chinense. (19, 25) Fagomine, a polyhydroxylated piperidine alkaloids previously reported in leaves and roots of Morus spp. (Moraceae) and leaves of Xanthoceras zambesiaca (leguminosae), was found to potentiate antihyperglycemic effect in streptozocin-induced diabetic mice and immunoreactive effect on insulin release.

Some papers published in Chinese journal have discussed the possible anti-diabetic constituents of Lycii Cortex. Zhou et al. (2002) revealed that the aqueous soluble constituents with low molecular weight were responsible for the hypoglycemic activity of Lycii Cortex, while, some hydro-soluble large molecules (supposed to be polysaccharides) displayed no effect on blood glucose (26). Through the TLC identification, the effective constituents were supposed to be alkaloids and

| Table 1. Summary of quantitative analysis for Lycii Cortex |
|-----------------|-----------------|-----------------|-----------------|
| **Analyte**     | **Method used** | **Content**     | **Literature**  |
| Vanillic acid   | HPLC-DAD        | 4.43-103.47 μg g⁻¹ | Li, et al., 2005; Li, et al. 2004 |
| Scopoletin      | HPLC-UV         | 52.4-99.3 mg g⁻¹ | Chu, et al., 2005 |
| Kaempferol      | Coupled with     | 25.0-38.8 mg g⁻¹ | Cai, et al., 2007 |
| Coumaric acid   | Capillary        | 18.3-27.8 mg g⁻¹ | Li, et al., 2005 |
| Vanillic acid   | electrophoresis | 16.3-34.8 mg g⁻¹ | Li, et al., 2005 |
| Luteolin        | Coupled with     | 9.2 mg g⁻¹ | Cai, et al., 2007 |
| Quercetin       | amperometric    | 9.9-10.0 mg g⁻¹ | Li, et al., 2005 |
| Betaine         | Detection        | 5.4-10.1 mg g⁻¹ | Li, et al., 2005 |
| Scopoletin      | HPLC-ELSD       | 17.0-1188.0 μg g⁻¹ | Li, et al., 2005 |
| Cinnamic acid   | HPLC-UV         | 2.2-51.7 μg g⁻¹ | Li, et al., 2005 |
peptides. Li et al. (2005) suggested that the acid fraction of Lycii cortex can significantly decrease blood glucose level in diabetic mice. However, the alkaline fraction had no significant effect. The neutral extract which was supposed to contain polysaccharides was toxic for animals. Anti-hypertensive effects

Funayama et al. (1980) revealed that methanol extract of Lycii cortex (as 0.5 g crude drug/Kg, i.v) produced significant hypotension in rats. Through repeated chromatography guided by hypotensive activity, kukoamine A (23) was isolated and confirmed to be responsible for antihypertensive activity (5 mg/Kg, i.v.).

Yahara et al. (1993) revealed that octapeptides, lyciumins A (33) and B (34), possessed significant renin and angiotensin converting enzyme (ACE) inhibition activities. Besides, the two fatty acids α-dimorphic acid (56) and 9-hydroxy-10,12,15-octadecatrienoic acid (57) were also reported to have anti-ACE activity.

Anti-inflammatory effects

Liu et al. (2011) used biosensor based screening technique for discovering anti-sepsis TCM. Lipopolysaccharides (LPS) and CpG DNA, two important pathogenic molecules and drug targets for sepsis, were immobilized on surfaces of biosensor for screening. Lycii cortex aqueous extract was selected out and found to have highest affinities to the dual-targets. Through bioactive-guided separation, kukoamine B (24) was purified and considered to be responsible for the activity. Monomer of kukoamine B can neutralize LPS and CpG DNA in vitro. It inhibited TRL4, TLR9 and MyD88 mRNA expressions, which can be up-regulated by LPS and CpG DNA. Additionally, kukoamine B can also alleviate NF-kB p65 protein in RAW 264.7 cells elicited by CpG DNA and LPS. The in vivo experiment showed that kukoamine B can protect mice from lethal challenge of heat-killed E. coli.

A comparison of the inhibitory effect between kukoamine B and polymyxin B (PMB) was performed. It was found that although kukoamine B have lower affinity to LPS than PMB, its LPS-neutralization capability was similar to that of PMB. Most importantly, the affinity to CpG DNA was only found in kukoamine B instead of PMB. In the cellular tests, kukoamine B was found to specifically inhibit TNF-α and IL-6 release instead of other pathogens. It was also found that the effect of kukoamine B to LPS and CpG DNA was through inhibiting their binding to macrophages. The direct neutralization of LPS and CpG DNA by kukoamine B was speculated as the main mechanism. This research group also compared the possible effective dose of kukoamine B with the commonly used anti-sepsis drugs, recombinant human activated protein C (rhAPC) and hydrocortisone. It was found that kukoamine B was in the similar order of magnitude to the clinically recommended dose of rhAPC and in lower dose than that of hydrocortisone. Therefore, kukoamine B could be considered as a candidate for treatment of sepsis.

The inhibitory activity was investigated on soybean lipoxigenase (LOX) and lipid peroxidation for kukoamine A and analogues. It was found that kukoamine A can significantly inhibit LOX with IC₅₀ of 9.5 μM. All tested analogues inhibited lipid peroxidation in the range of 11-100%. Kukoamine A was found to have comparable activity to indomethacin in anti-inflammatory activity with in vivo rat paw edema model induced by carrageenan.

Anti-parasites disease

Ponasik et al. (1995) have revealed that kukoamine A, an anthyptensive agent, can inhibit the enzyme trypanothione reductase (TR), an essential role in protecting parasitic trypanosomes against oxidative stress, however, it showed no significant inhibition of human glutathione reductase. Thus, kukoamine A provides a novel selective anti-parasitic drug lead.

Antimicrobial activity

(+)-lyoniresinol 3α-O-β-D-glucopyranoside (47) isolated from an ethyl acetate extract of Lycii cortex exhibited potent antimicrobial activity against antibiotic-resistant bacterial strains, methicillin-resistant Staphylococcus aureus (MRSA) isolated from patients, and human pathogenic fungi without having any hemolytic effect on human erythrocytes. In particular, this compound induced the accumulation of intracellular trehalose on C. albicans as stress response to the drug, and disrupted the dimorphic transition that forms pseudo-hyphae caused by the pathogenesis. Bis, tris and tetra (dihydrocaffeoyl) polyamine analogues showed antibacterial activity against vancomycin-resistant S. aureus (VRSA) with better effect than the reference drugs, vancomycin.

Anti-fungal activity

Phenolic amides (28-31) isolated from an ethyl acetate extract of CL presented anti-fungal effects. Compound 28-30 were potent at 5-10 μg mL⁻¹ and without hemolytic activity against human erythrocyte cells. Compound 31 was active at 40μg mL⁻¹.

Anti-oxidant activity

The ethyl acetate soluble fraction of Lycii Cortex was found to inhibit superoxide radical generation significantly in vitro. By means of a bioassay-directed chromatographic separation technique, three compounds, dihydro-N-caffeoyltartramine (28), trans-N-caffeoyltartramine (29) and cis-N-caffeoyltartramine (31) showed antioxidative activity in an NBT superoxide scavenging assay. The flavonoids contained in the LC, including quercetin, kaemperol, and myricetin may also be responsible for the activity.

Antitumor activity

Alkaloids 21 and 22 isolated from the root bark of LC had IC₅₀ value of 6.5 and 45 μg mL⁻¹ against glycosidase, respectively, and could be used as neoplasms inhibitors. It is also reported that scopoletin (48) also show activity to inhibit prostate carcinoma (PC3) cell proliferation.

CONCLUSION

Lycii cortex, as a Chinese traditional medicine, has been reported to exert various biological effects based on animal and cell studies. It has been confirmed that it possesses anti-diabetic, anticancer, and anti-inflammatory activity. However, kukoamine A and kukoamine B are the major bioactive compounds that may contribute to its biological activities. Although the biological effects are well-established, underlying molecular mechanisms are not fully understood. Therefore, it is highly recommended to do further research on the mechanism of kukoamine A and kukoamine B in various physiologic pathways.

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Pharmacy Study and Research Tour with China Pharmaceutical University

CHAN, Mei Kwan; NGAI, Vivian Cheuk Yan; NG, Freddie Ting Fat; WU, Suki Hoi Man
Department of Pharmacy & Pharmacology, University of Hong Kong, Pokfulam, Hong Kong

Abstract

Bachelor of Pharmacy students, along with teaching and research staff of Department of Pharmacology and Pharmacy, University of Hong Kong (HKU) participated in a one-week study and research tour hosted by China Pharmaceutical University (CPU). The objective of this exchange was to encourage both students and staff to exchange their knowledge and experience in clinical practice, teaching and research, to foster future partnership and collaboration. In this report, we share our experience from our visit to the main centres of CPU and the surrounding pharmaceutical sites.

Keywords: Pharmacy, pharmaceutical, study tour, China Pharmaceutical University, Chinese medicine

INTRODUCTION

The first partnership with China Pharmaceutical University 中國藥科大學 (CPU) was established this year. This programme aimed to provide a platform for pharmacy professors and students from the two schools to interact, establish friendships and exchange knowledge & experience at a regional level. Thirty two students from the University of Hong Kong (HKU) participated in this one-week exchange program hosted by CPU. In this short trip, a plethora of activities was meticulously organised for us to explore the world of pharmacy in mainland China. We visited 3 cities, namely Nanjing (南京), Wuxi (無錫), and Shanghai (上海), and had the opportunity to explore the four major areas in pharmacy, namely community, hospital, pharmaceutical industry and academia.

About CPU

China Pharmaceutical University 中國藥科大學 (CPU) is the leading pharmacy school in China, which boasts their high-ranking Pharmacy and Traditional Chinese Pharmacy Programmes. Established in 1936, CPU was part of the '211 project', which was initiated to raise the standards of research standards and enhance national Chinese economic and social development strategies. CPU currently has approximately 1000 faculty members, dedicated to teaching and research. The university offers a comprehensive range of bachelor degree programmes of different specialisations, from pharmaceutical engineering, food quality and safety, clinical pharmacy, traditional Chinese pharmacy, marine pharmacy, to international economics and trade (pharmaceutical). Within these specialisations, they offer short-term training courses, as well as master, doctoral, post-doctoral programmes. These programmes are offered and supported by the six schools within CPU, namely the School of Pharmacy, the School of Traditional Chinese Pharmacy, the School of Life Sciences & Technology, the School of International Pharmaceutical Business, the School of Continuing Education, and the Higher Vocational School of Pharmacy.

There are also four other departments within CPU, they are the Department of Basic Sciences, Department of Foreign Languages, Department of Social Sciences, and Department of Physical Education. These departments support CPU students with the aim to nurture them to become all-rounders aside from their specialised trainings within their chosen major disciplines. At the national level, there are three major national fields of pursuit, which target advancement in Medicinal Chemistry, Pharmacognosy and Pharmaceutics. The two key provincial fields focus on Modern Traditional Chinese Pharmacy and Pharmacognosy.

CPU is actively conducting research, having undertaken approximately 70 projects sponsored by the State Key Research Programmes Foundation, and more than 300 projects sponsored by the National Natural Sciences Foundation of China and the State New Drug Research Foundation. CPU is divided into the Xuanwumen Campus (玄武門校區) and Jiangning Campus (江寧校區), both situated in Nanjing, a dynamic and picturesque city. Our visit was primarily at the Jiangning Campus.

Laboratory sessions at CPU

The curriculum in CPU places emphasis on scientific research, and their students carry out pharmacology practicals using live animals. CPU students participated in all stages of the experiment, including anaesthetising the animal and collecting the required specimen. Animals used for undergraduate practicals included mice, guinea pigs and rabbits. This was a new experience for us all because, in contrast to CPU, we seldom use live animal experimentation in pharmacology practicals and supplement with the use of simulated computer models.

A warm welcome from CPU on arrival at the Jiangning Campus
A visit to the pharmaceutical museum in CPU

The pharmacy programme in CPU also places emphasis on the use of traditional Chinese herbs, as featured in a large pharmaceutical museum, showcasing a large variety of herbal medicines and the origins of medicinal compounds. Not all of the museum is opened to the multitude normally. Yet, with the approval from the university, we were privileged to be the first group of visitors to be allowed to view its entire, extensive collections. The museum was located at the central position of the school campus, comprises of three levels. The first floor would be open to the general public by appointment only; whereas the second and the third levels are restricted to CPU staff and students for educational purposes only.

The exhibition on the first floor focused on the history of Traditional Chinese Medicine (TCM) and how TCM evolved in Chinese history. Invaluable archaeological items and information relating to the artifacts were showcased. There was also a mock ancient Chinese pharmacy illustrating the set-up in ancient China.

The second and third floor of the museum were for the collections of different materials employed in preparing TCM and Western medicine. The second level places the focus on herbal plants, and even showcases a variety of species within genera. Not only did the exhibition show common plant products, it also incorporated relatively rare and costly ones such as Cordyceps (冬虫夏草) which is commonly the focus of research in the key state laboratories. On the third floor, TCM samples of plant, animal and mineral origins were exhibited. There was also a section on marine pharmacology and western medicines that were derivatives from marine species.

It was a great opportunity for pharmacy students to learn about this large collection of medicine-related specimen from the experts. With the help from the staff member of CPU, we have gained a better insight into how traditional and newly discovered materials found in Mother Nature ties in with modern pharmaceuticals and industrial processes.

A visit to the herb garden of CPU

In the afternoon, we were greeted by a large group of undergraduate students of CPU. They expressed their kind hospitality by pairing up with us on a one-to-one basis, and accompanied us on our visit to the herb garden.

Situated in the Jiangning campus, the herb garden is a treasure trove of beautiful botanical diversity. This tour to the garden gave us another close encounter with Chinese herbs. The herb garden is rather similar to our Hong Kong Park in Admiralty which is also filled with an amazing variety of plant species. Our “personal guide”, who were also experts in the use of traditional herbs, gave us a lot of information on whichever plant we directed our fingers at! Such a spectacular garden, quietly tucked in the back of the campus, possesses an atmosphere of tranquillity and it is mesmerising simply walking through. This made us wonder how stimulating it must be for TCM students to be able to gain first-hand knowledge about Chinese herbs outside the classroom.

Evening of exchange and performances

The CPU students and staff greeted us with great enthusiasm and tremendous cordiality on our first evening. In the theme of Pharmaceuticals and TCM, the students of CPU performed for us, displaying their multi-dimensional talents, from rapping, dancing, singing, to poetry recitation and martial arts. Two HKU students were even invited on stage to do a duet with them in singing and poetry.

HKU teachers and students shared some of our views and information on the subject of pharmacy education with our host by carrying out an impromptu problem-based learning (PBL) session, which is a unique teaching method adapted in HKU Medical Faculty. Selected students from both schools were given the opportunity to participate and interact with each other in a mock PBL session.

A vocal performance of a Tibetan folk song marked an end to our night of ringing laughter. At the end of the performance, each of us was presented with a khata, a traditional Tibetan offering scarf. Their kind hospitality shown throughout our stay is deeply appreciated and new friendship is formed.

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A new perspective in Chinese medicine

One of the major objectives for this trip was to encourage sharing of research interests and experiences. It was a great honour for us to attend a seminar co-organized by professors from both HKU and CPU. In the seminar, the Deans from three of the six schools and our professors presented their research interests and findings. The seminar was very inspiring. We were amazed by the sophisticated methods used to investigate the properties and therapeutic effects of the Chinese herbs. We also learnt to appreciate the challenges faced by researchers in Chinese medicine – TCM prescriptions are usually composed of various herbs in combination, as they often exhibit synergistic effects when used together. Purification of each ingredient for the study of its therapeutic effects may not be useful, as its behaviour may be altered when used together with another ingredient.

Life of pharmacy students in CPU and HKU

In our visit to CPU, we were so grateful to have a chance to have close interactions with CPU students. We realised that Pharmacy students in CPU and HKU share some similarities but also are different in many ways.

1. Study Programmes
The current HKU BPharm programme emphasises on pharmacy being an integration of a number of pharmacy-related studies; programmes of such nature are also available in CPU, but it also allows students to specialise by surveying many other programmes that focus on a particular subject, for example pharmacognosy and clinical pharmacy. The pharmacy school in CPU also offers bachelor courses specialising in areas such as pharmaceutical trade marketing and pharmaceutical management, which are not available in Hong Kong. For the undergraduate pharmacy programme in CPU, one striking difference with the one in HKU is that the curriculum offered in CPU has an emphasis on scientific research. Students are required to perform vast amounts of laboratory workshops ranging from animal experiments for pharmacology to the synthesis of various drugs in medicinal chemistry, and conducting field work in the pharmaceutical plant garden.

There are currently about 300 CPU students major in Pharmacy. Due to the relatively large class size in each year, they usually have lessons in lecture halls together with students major in other disciplines and seldom have small group tutorials as what we have in HKU.

2. Campus Lives
Since students in CPU are from different provinces, many of them apply for the residential halls located on the school campus. They have established a student association, which would arrange activities for the members. Similar as those in Hong Kong, hobbies of CPU students are multifarious such as sports, reading, watching television/drama and playing computer games.

3. Career Paths
Several CPU students revealed to us that many would like to work in the pharmaceutical industry after graduation for such enterprises in the mainland emphasise on drug research and development, to which they could apply what they learned throughout their study. In contrast to pharmacy graduates in Hong Kong, where many of us might work in hospitals or in the community, CPU students seem to have different career goals. The CPU students are convinced that the future would be promising in light of the prosperous growth of the pharmaceutical industry in China in recent years, as would the job market.

A visit to the Chinese Hospital

Apart from visiting CPU, we were arranged to visit the Nanjing Hospital of TCM which was a remarkable experience. Here, TCM plays a major role and is widely trusted by the patients. TCM techniques such as acupuncture were considered first line treatment for a wide variety of diseases, and there are wards dedicated for acupuncture. A few of us even volunteered to experience acupuncture by the therapists.

Tablets, Capsules and Herbs

Stepping into the Hospital Pharmacy, we were astonished by its setting. Instead of pills and capsules, the shelves were stacked with packets of herbs. Dispensers pack and dispense herbs to the patients according to the doctor’s prescription, and provide them with instructions for making the herbal tea. This is quite different from the practice in western medicine, where ready-to-consume medicines are usually dispensed. However, one may expect that there may be patients who lack the ability to prepare their own medicines, or simply do not have enough time for the lengthy boiling process. Having taken this into account, there is a preparation room in the Hospital where herbal tea is boiled and bottled. We have not seen such facilities in Hong Kong.
Visit to AstraZeneca

We arrived at AstraZeneca Wuxi site after a 2 hour coach ride from Hotel at Nanjing. Located in Wuxi New District, Jiangsu province, the site operates at a large scale, covering a total area of 96000 square meters. Furnished with first-rate manufacturing equipment, advanced injection solution plant, and efficient production line, Wuxi site also has high GMP standards. Before the start of the tour, we were instructed to remove our accessories such as earrings, necklaces, watches, and put on lab coats, shoe covers, and hair covers. We were most impressed by the sophisticated automation and organisation. Finished goods and raw materials can be stored and retrieved just by the click of a button on the computer. The temperature and humidity of the warehouse are closely monitored by thermostats and sensors installed. The cutting-edge infrastructure definitely left us in awe. It is of no wonder that the Wuxi site is capable of producing 95% of AstraZeneca products in the mainland market, covering a broad range of therapeutic areas like cardiovascular, gastrointestinal, respiratory, neuroscience, cancer, and infection, just to name a few.

CONCLUSION

Our brief experience in different aspects of pharmacy has given us new inspirations and thoughts. The warm hospitality that our host exuded has also made the visit very memorable and unforgettable. We look forward to future collaborations by establishing CPU as a permanent overseas exchange partner as that will definitely create unique memories and experiences for any participating student.

ACKNOWLEDGEMENT

Once again we would like to extend our deepest gratitude to CPU and HKU for all the arrangements, making our trip a remarkable experience. We would like to thank CHAN Chun Kit and CHEN Lu (pharmacy students, University of Hong Kong) for their contribution of photographs taken on the exchange tour. We thank Ms Phoebe Chan and Dr Esther Chan for their contributions to the manuscript.

Author’s background
CHAN, Mei Kwan, NGAI, Vivian Cheuk Yan, NG, Freddie Ting Fat, and WU, Suki Hoi Man are Bachelor of Pharmacy students; Chan, Phoebe Wing Lam is teaching consultant and Chan, Esther W is Assistant Professor Department of Pharmacy & Pharmacology, Faculty of Medicine, University of Hong Kong. Corresponding author’s email address is ewchan@hku.hk

Visit to AstraZeneca Wuxi site
Group photo taken after visit to AstraZeneca Wuxi site
BRINAVESS
(MERCK SHARP & DOHME (ASIA) LIMITED)

Product Name
BRINAVESS Concentrate for Solution for Infusion

Presentation:
Single-use glass vials each 25 ml containing 500 mg of vernakalant hydrochloride which is equivalent to 452.5 mg of vernakalant free base.

Pharmacology:
Vernakalant is an antiarrhythmic medicine that acts preferentially in the atria to prolong atrial refractoriness and to rate-dependently slow impulse conduction. These anti-fibrillatory actions on refractoriness and conduction are thought to suppress reentry, and are potentiated in the atria during atrial fibrillation. The relative selectivity of vernakalant on atrial versus ventricular refractoriness is postulated to result from the block of currents that are expressed in the atria, but not in the ventricles, as well as the unique electrophysiologic condition of the fibrillating atria. However, blockade of cardiac voltage-dependent sodium channels, which are present in the ventricles, has been documented.

Indications:
Rapid conversion of recent onset atrial fibrillation to sinus rhythm in adults.

Dosage and Administration:
BRINAVESS should be administered by intravenous infusion with an infusion pump. However, a syringe pump is acceptable provided that the calculated volume can be accurately given within the specified infusion time. Do not administer as an intravenous push or bolus.

A well-qualified healthcare professional should continuously monitor the patient during and for at least 15 minutes after the completion of the infusion.

BRINAVESS is dosed by patient body weight, with a maximum calculated dose based upon 113 kg. The recommended initial infusion is 3 mg/kg to be infused over a 10 minute period. For patients weighing ≥ 113 kg, do not exceed the maximum initial dose of 339 mg (84.7 ml of 4 mg/ml solution).

If conversion to sinus rhythm does not occur within 15 minutes after the end of the initial infusion, a second 10 minute infusion of 2 mg/kg may be administered. For patients weighing ≥ 113 kg, do not exceed the maximum second infusion of 226 mg (56.5 ml of 4 mg/ml solution). Cumulative doses of greater than 5 mg/kg should not be administered within 24 hours.

If conversion to sinus rhythm occurs during either the initial or second infusion, that infusion should be continued to completion. If hemodynamically stable atrial flutter is observed after the initial infusion, the second infusion of BRINAVESS may be administered as patients may convert to sinus rhythm. (See PRECAUTIONS and SIDE EFFECTS.)

Preparation of BRINAVESS for infusion

Step 1: Visually inspect BRINAVESS vials for particulate matter and discoloration before administration. Do not use any vials exhibiting particulate matter or discoloration. BRINAVESS concentrate for solution for infusion ranges from colorless to pale yellow. Variations of color within this range do not affect potency.

Step 2: Dilution of concentrate
A sufficient amount of BRINAVESS 20 mg/ml should be prepared at the outset of therapy to deliver the initial and second infusion should it be warranted. Create a solution with a concentration of 4 mg/ml following the dilution guidelines below. Recommended diluents are 0.9% Sodium Chloride for Injection, Lactated Ringers for Injection, or 5% Dextrose for Injection.

Patients ≤ 100 kg: 25 ml of BRINAVESS 20 mg/ml is added to 100 ml of diluent.
Patients > 100 kg: 30 ml of BRINAVESS 20 mg/ml is added to 120 ml of diluent.

Step 3: Inspect solution
The diluted sterile solution should be clear, colourless to pale yellow. Visually re-inspect the solution for particulate matter and discoloration before administering. The diluted sterile concentrate is chemically and physically stable for 12 hours at or below 25°C. However, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C to 8°C, unless dilution has taken place in controlled and validated aseptic conditions.

Discard any unused product or waste material.

Method of administration
BRINAVESS vials are for single use only and must be diluted prior to administration.

Step 4: Administration of the initial infusion
The initial infusion of BRINAVESS is administered as a 3 mg/kg dose over 10 minutes.

Step 5: Patient observation
If conversion to sinus rhythm has not occurred, observe the patient’s vital signs and cardiac rhythm for an additional 15 minutes.

Step 6: Administration of second infusion
If conversion to sinus rhythm did not occur with the initial infusion or within the 15 minute observation period, administer a 2 mg/kg second infusion over 10 minutes.

Post-cardiac surgery patients: No dose adjustment necessary.
Renal impairment: No dose adjustment necessary.
Hepatic impairment: No dose adjustment necessary.
Elderly (≥ 65 years): No dose adjustment necessary.
Children and adolescents (< 18 years old): There is no experience on the use of BRINAVESS in children and adolescents (< 18 years); therefore its use is not recommended in this population.

Contraindications:
Patients with hypersensitivity to vernakalant hydrochloride or to any of the excipients.
Patients with severe aortic stenosis, systolic blood pressure <100 mm Hg, and heart failure class NYHA III and NYHA IV.
Patients with prolonged QT at baseline (uncorrected > 440 msec), or severe bradycardia, sinus node dysfunction, or second degree or third degree heart block in the absence of a pacemaker.
Patients using intravenous rhythm control anti-arrhythmics (class I and class III) within 4 hours prior to BRINAVESS administration.

Patients with acute coronary syndrome (including myocardial infarction) within the last 30 days.

Warnings:
BRINAVESS should only be administered by qualified medical personnel in a monitored clinical setting appropriate for cardioversion. Patients should be observed with assessment of vital signs and continuous cardiac rhythm monitoring during and after administration of BRINAVESS, until clinical and ECG parameters have stabilised. Continuous monitoring of blood pressure is also required during and at least 15 minutes after the completion of the infusion.
Direct-current cardioversion may be considered for patients who do not respond to therapy.

Prior to attempting pharmacological cardioversion, ensure that patients are adequately hydrated and haemodynamically optimized and if necessary patients should be anticoagulated. In patients with uncorrected hypokalaemia (serum potassium of less than 3.5 mmol/l), potassium levels should be corrected prior to use of BRINAVESS.

During infusion of BRINAVESS, if a patient develops clinically meaningful bradycardia, has an unexpected drop in blood pressure, becomes hypotensive, or develops ECG changes (such as a clinically meaningful sinus pause, complete heart block, new bundle branch block, significant prolongation of the QRS or QT interval, changes consistent with ischemia or infarction and ventricular arrhythmia), the administration of BRINAVESS should be discontinued. If these events occur during the first infusion of BRINAVESS, patients should not receive the second dose of BRINAVESS.

Hypotension
Hypotension typically occurs early, either during the infusion or early after the end of the infusion. Patients with congestive heart failure (CHF) have been identified as a population at higher risk for hypotension.

Congestive Heart Failure
Patients with CHF showed a higher overall incidence of hypotensive events and ventricular arrhythmia during the first 2 hours after dose in patients treated with vernakalant compared to patients receiving placebo. These arrhythmias typically presented as asymptomatic, monomorphic, non-sustained (average 3-4 beats) ventricular tachycardias.

Vernakalant should be used cautiously in haemodynamically stable patients with CHF functional classes NYHA I to II. There is limited experience with the use of vernakalant in patients with previously documented LVEF 35%. Its use in these patients is not recommended.

Atrial Flutter
BRINAVESS was not found to be effective in converting typical primary atrial flutter to sinus rhythm. Patients receiving BRINAVESS have a higher incidence of converting to atrial flutter within the first 2 hours post-dose. This risk is higher in patients who use Class I antiarrhythmics. If atrial flutter is observed as secondary to treatment, continuation of infusion should be considered.

Use of AADs (anti-arrhythmic drugs) prior to or after BRINAVESS
BRINAVESS can not be recommended in patients previously administered intravenous AADs (class I and III) 4-24 hours prior to vernakalant. BRINAVESS should not be administered in patients who received intravenous AADs (class I and III) within 4 hours prior to vernakalant.

BRINAVESS should be used with caution in patients on oral AADs (class I and III). Risk of atrial flutter may be increased in patients receiving class I AADs (see above).

There is limited experience with the use of intravenous rhythm control anti-arrhythmics (class I and class III) in the first 4 hours after BRINAVESS administration, therefore these agents should be used cautiously within this period. Resumption or initiation of oral maintenance antiarrhythmic therapy can be considered starting 2 hours after vernakalant administration.

Valvular Heart Disease
In patients with valvular heart disease, there was a higher incidence of ventricular arrhythmia events in vernakalant patients. These patients should be monitored closely.

Furthermore, BRINAVESS has not been evaluated in patients with clinically meaningful valvular stenosis, hypertrophic obstructive cardiomyopathy, restrictive cardiomyopathy, or constrictive pericarditis and its use can not be recommended in such cases. There is limited experience with BRINAVESS in patients with pacemakers.

As the clinical trial experience in patients with advanced hepatic impairment is limited, vernakalant is not recommended in these patients.

Each vial of 500 mg contains approximately 3.5 mmol (80 mg) of sodium. This should be taken into consideration by patients on a controlled sodium diet.

When driving vehicles or operating machines, it should be taken into account that, dizziness has been reported within the first two hours after taking BRINAVESS.

Interaction
No formal interaction studies have been undertaken with vernakalant injection.

Although vernakalant is a substrate of CYP2D6, no substantial differences in the acute exposure of vernakalant were observed when weak or potent CYP2D6 inhibitors were administered within 1 day prior to vernakalant infusion compared to patients that were not on concomitant therapy with CYP2D6 inhibitors. In addition, acute exposure of vernakalant in poor metabolizers of CYP2D6 is only minimally different when compared to that of extensive metabolizers. No dose adjustment of vernakalant is required on the basis of CYP2D6 metabolizer status, or concurrent administration with 2D6 inhibitors.

Vernakalant is a moderate, competitive inhibitor of CYP2D6. However, acute intravenous administration of vernakalant is not expected to markedly impact the PK of chronically administered 2D6 substrates.

Pregnancy and lactation:
Use in pregnancy: There are no data from the use of vernakalant hydrochloride in pregnant women. As a precautionary measure, it is preferable to avoid the use of vernakalant during pregnancy.

Use in lactation: It is unknown whether vernakalant/ metabolites are excreted in human milk. Caution should be exercised when used in breastfeeding women.

Side effects:
The most commonly reported side effects (> 5%) seen in the first 24 hours after receiving BRINAVESS were dysgeusia (taste disturbance), sneezing and paraesthesia.

The frequencies of main adverse reactions are expressed in patient-years, according to the following categories: very common ≥ 1/10, common 1/100 to < 1/10, uncommon ≥ 1/10,000 to < 1/100, rare < 1/100,000.

Nervous System Disorders:
Very common: Dysgeusia.
Common: Paraesthesia, dizziness, headache, hypoaesthesia.
Uncommon: Burning sensation, parosmia, somnolence, vasovagal syncope.

Eye disorders:
Uncommon: Eye irritation, lacrimation increased, visual disturbance.

Cardiac disorders:
Common: Bradycardia, atrial flutter.
Uncommon: Sinus arrest, first degree AV block, left bundle branch block, ventricular extrasystoles, palpitations, sinus bradycardia, ventricular tachycardia, ECG QRS complex prolonged, ECG QT prolonged, cardiogenic shock.

Vascular disorders:
Common: Hypotension.
Uncommon: Flushing, hot flush, pallor.

Respiratory, thoracic and mediastinal disorders: Very common: Sneezing.
Common: Coughing, nasal discomfort.
Uncommon: Dyspnea, suffocation feeling, rhinorrhea, throat irritation.

Gastrointestinal Disorders:
Common: Nausea, vomiting, dry mouth.
Uncommon: Diarrhoea, defecation urgency.

Skin and subcutaneous tissue disorders:
Common: Pruritus, hyperhidrosis.
Uncommon: Generalized pruritus, cold sweat.
**Pneumococcal polysaccharide**

**serotype 4**  2.2

**serotype 3**  2.2

Based on serotype surveillance in Europe performed before the introduction of Prevenar, Prevenar 13 is estimated to cover 73-100 % (depending on the country) of serotypes causing invasive pneumococcal disease (IPD) in children less than 5 years of age. In this age group, serotypes 1, 3, 5, 6A, 7F, and 19A account for 59.7 % of invasive disease, depending on the country, the time period studied, and the use of Prevenar.

**Indications:**

Active immunisation for the prevention of invasive disease caused by *Streptococcus pneumoniae* in adults aged 50 years and older.

**Dosage & Administration:**

The vaccine should be given by intramuscular injection.

The immunisation schedules for Prevenar 13 should be based on official recommendations. It is recommended that infants who receive a first dose of Prevenar 13 complete the vaccination course with Prevenar 13.

**Three-dose primary series**

The recommended immunisation consists of four doses, each of 0.5 ml. The primary infant series consists of three doses, with the first dose usually given at 2 months of age and with an interval of at least 1 month between doses. The first dose may be given as early as six weeks of age. The fourth (booster) dose is recommended between 11 and 15 months of age.

**Two-dose primary series**

Alternatively, when Prevenar 13 is given as part of a routine infant immunisation programme, a series consisting of three doses, each of 0.5 ml, may be given. The first dose may be administered from the age of 2 months, with a second dose 2 months later. The third (booster) dose is recommended between 11 and 15 months of age.

**Forensic Classification:**

**P1S1S3**

**Active ingredients:**

1 dose (0.5 ml) contains:

- Pneumococcal polysaccharide serotype 1  2.2 µg
- Pneumococcal polysaccharide serotype 3  2.2 µg
- Pneumococcal polysaccharide serotype 4  2.2 µg
- Pneumococcal polysaccharide serotype 5  2.2 µg
- Pneumococcal polysaccharide serotype 6A  2.2 µg
- Pneumococcal polysaccharide serotype 6B  4.4 µg
- Pneumococcal polysaccharide serotype 7F  2.2 µg
- Pneumococcal polysaccharide serotype 9V  2.2 µg
- Pneumococcal polysaccharide serotype 14  2.2 µg
- Pneumococcal polysaccharide serotype 18C  2.2 µg
- Pneumococcal polysaccharide serotype 19A  2.2 µg
- Pneumococcal polysaccharide serotype 19F  2.2 µg
- Pneumococcal polysaccharide serotype 23F  2.2 µg

**Presentation:**

0.5 ml suspension for injection in pre-filled syringe

**Pharmacological Properties:**

Prevenar 13 contains the 7 pneumococcal capsular polysaccharides that are in Prevenar (4, 6B, 9V, 14, 18C, 19F, 23F) plus 6 additional polysaccharides (1, 3, 5, 6A, 7F, 19A) all conjugated to CRM197 carrier protein.

**Side Effects:**

Decreased appetite; pyrexia; irritability; any injection-site erythema, induration/swelling or pain/tenderness; somnolence; poor quality sleep; injection-site movement impairment (due to pain); apnoea in very premature infants (< 28 weeks of gestation).

**Forensic Classification:**

P1S1S3

**Active ingredient:**

Domperidone

**Presentation:**

10 mg film-coated tablets and 1mg/ml oral suspension

**Pharmacological Properties:**

Domperidone is a dopamine antagonist with anti-emetic properties

**Indications:**

Dyspeptic symptom complex associated with delayed gastric emptying, gastro-oesophageal reflex & oesophagitis. Nausea & vomiting of functional, organic, infectious or dietetic origin, or induced by radiotherapy or drug therapy. Nausea & vomiting induced by dopamine agonists, as used in Parkinson’s disease.

**Dosage and Administration:**

Adult & ≥12 yr & ≥45 kg: 1-2 tablets 3-4 times daily with a maximum daily dose of 80 mg

Oral suspension: 10-20 ml 3-4 times daily with a maximum daily dose of 80 ml

Children >2 yr: 0.2-0.4 mg/kg every 4-8 hours
The world of PPIs is about to change.

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0.01%的可能性是以第三者觀察圖像測試作評估在其中88%男士頭髮增長，42%停止脫髮，68%以頭髮數量作評估。上述p值的為<0.001。這結果是根據

一項長達三年的臨床研究，1353位18-41歲有輕中至中度頭頂脫髮之男士，經由醫生診斷後安排服用保康絲或安慰劑，以評估保康絲之效果以頭髮數量，病人及醫生整體評估，第三者

觀察圖像查詢及安全


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