News & Short Communications

A Lecture Review on “Coaching – Practical Exercises, Discussion and Case Examples” by Charlie Lang (Hong Kong Pharmacy Conference 2016 Concurrent Session III)

An Overview of the Pharmaceutical Properties of Oral Care Products (2 CE Units)

Biogenesis Regulation and Detection Methods of MicroRNA Expression Profiling

Blood-tonifying and Regulating Functions of Paeoniae Radix (Shaoyao)

Forbidden City International Pharmacist Forum 2016 Pharmacy Students Report

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Reference:

Abbreviated Prescribing Information:
P/V: 300mg supplied as a lyophilized powder for dilution for infusion. C: Vedo boisn is for treatment of adult patients with moderate-to-severe active Crohn’s disease. A: Vedo boisn is for treatment of adult patients with active Crohn’s disease. D: For UC/CD. Entyvio 300mg IV infusion over 30 minutes at 0th, 2nd week and 8th week then every 8 weeks thereafter. For UC patients who experience a decrease in response or loss of response, the dose may be increased to every 6 weeks. For CD patients who have shown a response to Entyvio 300mg every 4 weeks, the dose may be increased to every 8 weeks. E: Hypersensitivity, active severe infections, opportunistic infections. S: Infusion-related reactions, infections, malabsorption, ileal and oral vaccines. A: Nasopharyngitis, bronchitis, gastritis, upper respiratory tract infection, inflammation, sinusitis, pharyngitis, headache, asthenia, hyperesthesia, upper respiratory tract infection, nasal congestion, cough, oral abscess, oral ulcer, nausea, dyspepsia, constipation, abdominal distension, flatulence, abdominal discomfort, rash, pruritus, eczema, dysgeusia, night sweats, acne, arthralgia, muscle spasm, back pain, musculoskeletal pain, tongue, pyrosis. D: No interaction studies have been performed.
Editorial
CHEUNG, Hon-Yeung  36

News & Short Communications
Methylphenidate Increases Risk of Arrhythmia for Young ADHD Patients  37
Amiodarone and Lidocaine are Ineffective in Out-of-Hospital Cardiac Arrest, Study Finds  37
Low-Dose Intravenous Alteplase is Safer But Not As Effective As Standard Dose  37
Study Doubts Superiority of Ticagrelor Acute Stroke or Transient Ischemic Attack  38
Health Supplements May Reduce the Need for ESBC Chemotherapy  38
Botox for Beauty can Cost More Than Imagined, Research Shown  38
SABA-LABA Combination is Proven Better than LABA-ICS in COPD Management  39
Liraglutide Added to High-Dose Insulin Therapy Improved Glycaemic Control  39
Canagliflozin: Signal of Increased Risk of Lower Extremity Amputations Observed in Trial in High Cardiovascular Risk Patients  40
New Medicine Labelling Requirements to Take Effect from August 5, 2016  40
Implementation Plan of Phase 2 Requirement of Bioavailability and Bioequivalence Studies for the Registration of Generic Drugs  41

Pharmacy Education & Practice
A Lecture Review on “Coaching – Practical Exercises, Discussion and Case Examples” by Charlie Lang  42
(Hong Kong Pharmacy Conference 2016 Concurrent Session III)
HUI, Wynne CW; CHONG, Donald WK

Drugs & Therapeutics
An Overview of the Pharmaceutical Properties of Oral Care Products (2 CE Units)  45
CHAN, Yi-Ming

Pharmacological Techniques & Technology
Biogenesis Regulation and Detection Methods of MicroRNA  51
Expression Profiling
ALQOUQA, Iyad; AL Zaharn, Mazen; CHEUNG, Hon Yeung

Herbal Medicines & Nutraceuticals
Blood-tionifying and Regulating Functions of Paeoniae Radix (Shaoyao)  59
ZHAO, Hang; LI, Linqiu; CHEUNG, Hon-Yeung

Society Activities
Forbidden City International Pharmacist Forum 2016 Pharmacy Students Report
Irene Chan, Rita Cheung, Matthew Ho Edward Lam, Tom Leung, Bentley Liu

New Products
GARDASIL 9  68
JARDIANCE®  69
The Professional Spirit underneath the White Coat

The following is my inspirational sharing with some freshmen admitted this summer to the Department of Pharmacology & Pharmacy in the University of Hong Kong. Hence, it is enclosed here for your perusal.

Good evening everyone and thank you for giving me this chance to speak to you tonight in your White Coat Ceremony. Coming here to speak to you is particularly meaningful to me since it was almost half a century ago that I was admitted to a pharmacy school like you and began my learning in pharmacy. It is really hard to believe that it has been 42 years since I was graduated. I emphasize learning because there are lots of new knowledge for me to learn every day. I have no regret so far throughout these years. Although I didn’t practice my profession three years after my graduation, I have been doing something close to pharmacy practice because I choose to do pharmaceutical research and education.

Pharmacist is a health care professional. Whoever in this profession, is entrusted with right and responsibility to handling, manufacturing, dispensing and merchandizing drugs or providing advice and information to clients on the use of therapeutics. Like many other health care professionals, mistake or error is intolerable during their work because it may affect a person’s life.

In order to become a competent and successful pharmacist, it is necessary to equip yourself with strict and very often tough trainings. Pharmacy courses, in general, are quite demanding and difficult. It is a professional requiring multidisciplinary knowledge. Hence, you are asked to do courses in biology, chemistry, organic chemistry, biochemistry, physical chemistry, microbiology, immunology, analytical chemistry, pharmacognosy, pharmacology, dispensary, pharmaceutics, medicinal chemistry, biotechnology, management and documentation of clinical data or pharmacy store, forensic law and even courses related to engineering for some specialized stream, such as industrial pharmacy … etc. There are really lots of things to learn and to master before you are allowed to practicing your profession independently. If you are not prepared in advance, you will definitely get lost. If you don’t know how to study, you will probably unable to do well and perhaps fail subsequently. When I did my pharmacy courses during the early 70s in Taiwan, one fourth of my classmates had to repeat or told to withdraw from the course after their first year of study because they failed in their course assessment.

The 1st important spirit to become professionalism is your determination to excellence in all that you endeavor.

Most people know that the hierarchy of capabilities consists of 6 level of categories of human thinking skills, namely, level (1) remembering; (2) understanding; (3) applying; (4) analyzing; (5) evaluating and (6) creating. Among all capabilities, remembering is at the bottom and learning is far more than just remembering. That learning is more important than memorizing. This statement is partially true if we emphasize acquiring new knowledge alone. But to apply knowledge for solving problem or for creative work, a prompt action derived from good memorized data is always favorable alone. But to apply knowledge for solving problem or for creative work, a prompt action derived from good memorized data is always favorable.

Besides, there are some other important matters for a college student to master: i.e. to delegate, to plan, to accept responsibility, to learn & to do pharmaceutical research and education.

The 2nd important spirit to become professionalism is your commitment to the highest standards of ethics.

In a book entitled “Professionalism, work, and clinical responsibility in pharmacy” Pharmacist David Tipton said the manner related to professionalism include punctuality, appearance, courtesy, concern for others, grace under pressure, honesty, discretion, judgment, and commitment to excel. These are issues all about your character. It is the generator (dynamo) of professionalism. Things like honesty, humility and sincerity are important. No one can give you these things, they don’t come automatically with a degree. You can’t borrow character, you have to own it, hold it and preserve it. A strong desire to nurture these good human characters is a must. Otherwise, you will be corrupted gradually.

The 3rd important spirit to become professionalism is your care and concern for other people needs and for the advancement of your profession.

True professionalism means showing that you really care. The fuel of professionalism is your care and concern for the maximal benefit of your patient and for the advancement of your profession.

You may ask why we have this situation in Hong Kong. Well, it is because some of us are not bothered to make advancements for what we are searching. They are afraid that their incomes may be temporary affected if they support the change. Therefore, they do their best to block the movement. This is purely a selfishness behavior and does not conform to the spirit of a profession. Let us be reminded, a profession won’t be established, prosperous or growth by itself unless everyone working in the field put in all efforts to nature its worthy of existence. On top of this, an organized professional society should have her outlet, such as academic journals or media platform to allow their members to voice out or to discuss whatever affairs affect their practices in a society. To this point, I would like to invite you to join and to support the Hong Kong Pharmaceutical Society, which is your professional home. And if possible, transform your ideas or thinking into written format in an article and get it published in HK Pharmaceutical Journal to show your support. It is only through a wider discussion that we can find out the best solution for our profession. It will certainly offer some immediate beneficial to you as it is a reflection of your intellectual achievement.

To close my talk, I would like to quote a statement made by Dr. G. Tiptoe, your Assistant Dean of the LKS Faculty of Medicine of HKU. He said at the beginning of the ceremony that this ceremony, although simple, is important particularly for you to signify your commitment to the profession of pharmacy. You will become a pharmacist in your life in about four years if you meet all requirements and I am quite sure you are excited. Whether you will be a good pharmacist or not depends very much on whether you own these three spirit or not.

Editorial

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The Professional Spirit in Hong Kong

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Methylphenidate Increases Risk of Arrhythmia for Young ADHD Patients

Date: April 25, 2016

The first-line drug treatment for attention-deficient/hyperactivity disorder (ADHD), methylphenidate, is effective in reducing symptoms of impulsivity and hyperactivity in children. However, it was found to be associated with sudden death.

A retrospective, self-controlled case analysis was conducted to investigate the link between possible adverse cardiovascular events and methylphenidate, utilizing the National Health Insurance Database of South Korea (from 2008 to 2011 inclusive). It included 1224 patients aged 17 or below with incidence of a cardiovascular event and newly prescribed methylphenidate. The incidence of adverse events was compared for each patient during exposure and non-exposure of methylphenidate. Arrhythmias, hypertension, myocardial infarction, ischemic stroke and heart failure were the targeted adverse events.

During methylphenidate treatment, the overall risk of arrhythmia increased (incidence rate ratio 1.61, 95% CI: 1.48 - 1.74), and the risk was the highest in children with pre-existing congenital heart disease and in the first 3 days of drug use. Risk of myocardial infarction was higher between 8 and 56 days since the treatment. No association was found between methylphenidate treatment and other adverse events (hypertension, ischemic stroke or heart failure).

It was concluded that the absolute risk was likely to be small. Although more prospective and observational studies are needed, caution should be exerted over the use of methylphenidate for children and adolescents with ADHD.

Source: www.bmj.com

Amiodarone and Lidocaine are Ineffective in Out-of-Hospital Cardiac Arrest, Study Finds

Date: May 5, 2016

A trial was conducted at 10 North American sites to compare the effectiveness of of parenteral amiodarone, lidocaine, and saline placebo in increasing the rate of survival or favorable neurologic outcome in adults with non-traumatic out-of-hospital cardiac arrest, shock-refractory ventricular fibrillation or pulseless ventricular tachycardia after at least one shock and vascular access.

The randomized, double-blind trial was conducted with 3026 patients, which were randomly assigned to amiodarone, lidocaine or placebo with standard care given along with the trial. The percentage of survival to hospital discharge was 24.4%, 23.7% and 21.0% respectively. As for neurologic outcome at discharge, the result was similar in the three groups.

It can be concluded that neither amiodarone nor lidocaine can result in significantly higher rate of survival or favorable neurologic outcome when compared to the result of that of the placebo-receiving patients with out-of-hospital cardiac arrest due to initial shock-refractory ventricular fibrillation or pulseless ventricular tachycardia

Source: www.nejm.org

Low-Dose Intravenous Alteplase is Safer But Not As Effective As Standard Dose

Date: May 10, 2016

Intravenous alteplase at a standard-dose of 0.9 mg per kilogram body weight is an effective thrombolytic therapy for acute ischemic stroke. The drawback of this treatment is an increased risk of intracerebral hemorrhage.

A randomized, open-label trial with quasi-factorial designed was planned in 2010 and completed in 2015 to compare the efficacy and safety of low-dose (0.6 mg per kilogram body weight) with standard-dose intravenous alteplase in patients with acute ischemic stroke. 3310 patients (63% Asian) in 13 countries were randomly assigned to receive either treatment within 4.5 hours after the onset of stroke. The primary outcome was death or disability at 90 days, defined by scores of 2 to 6 on the modified Rankin scale. The major secondary outcome was intracerebral hemorrhage, defined by that given in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study.

The low-dose group showed a higher occurrence of death or disability at 90 days than the standard dose group (53.2% vs 51.1%) and failed to show non-inferiority to the standard-dose group. Nevertheless, the occurrence of major symptomatic intra-cerebral hemorrhage was significantly lower in the low-dose group (1.0%) than the standard-dose group (2.1%). The percentage of fatal events occurred within 7 days was lower in the former (0.5%) than the latter (1.5%). No significant difference in the mortality at 90 days was observed between the two groups.

Low-dose intravenous alteplase was not non-inferior to standard-dose treatment for acute ischemic stroke; however, it showed significantly less risk of symptomatic intra-cerebral hemorrhage.

Source: www.nejm.org
Study Doubts Superiority of Ticagrelor Acute Stroke or Transient Ischemic Attack

Date: May 10, 2016

Ischemic stroke and transient ischemic attack are prevalent. Worse still, the risk of subsequent strokes within 90 days is high. To address the issue, a daily dose of aspirin (50-325mg) is commonly used. Nevertheless, the treatment shows a limited benefit and is associated with internal bleeding. Given these limitations, antiplatelet therapies with other mechanisms of action are studied, especially ticagrelor (potent P2Y12 receptor antagonist).

SOCRATES was a multi-centre, randomized, double-blind, double-dummy, parallel-group trial. It aimed to compare the efficacy of ticagrelor with aspirin to prevent major vascular events (stroke, myocardial infarction and death) over 90 days in patients with acute cerebral ischemia. 13,307 patients (aged 40 or above) with acute ischemic stroke were enrolled but 108 were excluded. Approximately half of the patients received ticagrelor (two 90-mg tablets on day 1, then 90 mg twice daily) while the other half received aspirin (a loading dose of 300 mg given as three 100-mg tablets on day 1, then 100 mg daily). The primary end point was the time for the occurrence of major vascular events after randomization. The secondary end points were time to ischemic stroke and other adverse events especially internal bleeding.

No significant difference was found between ticagrelor and aspirin groups for the primary end-point (hazard ratio: 0.89; 95% CI, 0.78 - 1.01, P = 0.07). Except for ischemic stroke (hazard ratio: 0.87; 95% CI, 0.76 - 1.00, P = 0.046), no significant difference was found for other secondary end-points. Although the rate of serious adverse events did not differ significantly between the two groups (hazard ratio, 0.83; 95% CI, 0.52 - 1.34), discontinuation of treatment due to dyspnea and any bleeding was more common in the ticagrelor group.

In conclusion, ticagrelor was found to be non-superior to aspirin in reducing the risk of major vascular events.

Source: www.nejm.org

Health Supplements May Reduce the Need for ESBC Chemotherapy

Date: May 12, 2016

Women with early-stage breast cancer for whom chemotherapy was indicated and who used dietary supplements and multiple types of complementary and alternative medicine (CAM) were less likely to start chemotherapy than the non-users, according to the latest research led by Heather Greenlee, associate professor of Epidemiology at Columbia University.

A group of 685 women with early-stage breast cancer were included from 2006 to 2010. Five types of complementary therapies, such as the dietary supplement use of vitamins, minerals, herbs and botanicals were being investigated.

The use of alternative therapies was reported by a considerable proportion (87%) of the women studied. By 12 months, chemotherapy was initiated by 89% of women for whom chemotherapy was indicated, while the remaining ones for whom chemotherapy was discretionary had a much lower rate of initiation (36%). Complementary and alternative therapy used among breast cancer patients has become more prevalent in the past two decades, with dietary supplements and mind-body practices being the commonest.

Greenlee suggests that there may be alternative explanations for their findings - it is unclear whether the association between CAM usage and chemotherapy non-initiation reflects long lasted decision-making patterns among study participants. Possibly the women who did not initiate treatment and who were alternative therapy users were long-time users of CAM and preferred complementary medicine to conventional chemotherapy.

Pharmacists should interpret the results carefully and it is beneficial to ascertain use of CAM therapy among their patients, so as to avoid adverse effects on patients undergoing chemotherapy.

Source: www.sciencedaily.com

Botox for Beauty can Cost More than Imagined, Research Shown

Date: May 12, 2016

Recently, increased numbers of hospitalization related to fake botox have sparked heated discussion. China Food and Drug Administration warned the public about unqualified medical institutions offering fake Botox injections, as a minimum of four Hong Kong women were reported sick after Botox treatment on the mainland in the past two months, according to Hong Kong’s Centre for Health Protection. A rising trend in the number of patient seeking for follow-up treatments at local dermatologists clinic after receiving beauty treatment in mainland China is seen, with a 20 to 30 percent increase, when compared with last year.

The injections are intended to make one’s skin appear younger as a result of a mild paralysis. Yet, beside the safety hazard, another unpredictable effect is found: they undermine people’s ability to understand others’ facial expressions by reproducing them on their own bodies, through a temporary block of proprioceptive feedback.

The perception of emotional information and facial expressions is mostly affected by botulin injections. Embodiment, a well known scientific theory states that the processing of emotional information, such as facial expressions, involves reproducing the same emotions on our own bodies. In other words, when we observe a smile, our face tends to smile too, often imperceptibly and automatically as we try to make sense of that expression. However, if our facial muscles are paralyzed by...
Botox, the process of understanding others’ emotion expression may become more difficult.

Jenny Baumeister, research scientist at the International School for Advanced Studies (SISSA), conducted different tests on subjects to assess their understanding of emotions immediately before and two weeks after they had Botulin injections. She compared them with those who had no treatment. Consequently, the effect of the paralysis was obvious. “The defect is very clear when the expressions observed are subtle.” explained Francesco Foroni, SISSA researcher who coordinated the study.

The finding also suggests that the drawback of Botox may be huge in situations in which understanding expressions are subtle. It may become more difficult to understand feelings in situations where mutual understanding is vital to ensure proper social interaction, failure to pick up on emotional nuances or sudden changes in the other person’s mood can make the difference between successful communication and communication breakdown.

While botox treatments may cause profound safety and health consequences, the Hong Kong Medical Association advised the public to learn about the functions and risks of the treatments before injection, and to have the botox injection procedures done by qualified doctors.

Source: www.sciencedaily.com and www=scmp.com

SABA-LABA Combination is Proven Better than LABA-ICS in COPD Management

Date: May 15, 2016

Current guidelines recommend the combination of long-acting beta-antagonist (LABA) and inhaled glucocorticoid as the primary treatment to control chronic obstructive pulmonary disease (COPD) exacerbations. However, long-term use of glucocorticoids would increase the risk of pneumonia and other adverse effects.

A research group has conducted a randomized, double-blind, non-inferiority trial to compare the efficacy of the combination of LABA and long-acting muscarinic antagonist (LAMA) with the standard primary treatment. COPD patients with a history of at least one exacerbation in the previous year were recruited in 43 countries. 1680 patients were assigned to receive LABA salmeterol 110 μg plus LAMA glycopyrronium 50 μg once daily. 1682 patients were assigned to receive LABA salmeterol 50 μg plus inhaled glucocorticoid fluticasone 500 μg twice daily. The medications were taken by inhalation for 52 weeks.

The primary outcome recorded was the annual rate of COPD exacerbations including all levels of severity. It was 11% lower in the indacaterol–glycopyrronium group (3.59) than the salmeterol–fluticasone group (4.03), demonstrating that the former was not only non-inferior but even superior to the latter. The indacaterol–glycopyrronium group performed better in other secondary outcomes measured. The annual rate of exacerbations with moderate or high severity was lower in the LABA-LAMA group (0.98) than the LABA-inhaled glucocorticoid group (1.19). The time to first exacerbation was longer in the LABA-LAMA group (71 days) than the LABA-inhaled glucocorticoid group (51 days). The two groups showed similar incidence of adverse events and deaths, while the indacaterol–glycopyrronium group showed a lower incidence of pneumonia than the salmeterol–fluticasone group (3.2% vs 4.8%).

In conclusion, the combined use of indacaterol and glycopyrronium could be a better alternative with higher efficacy than salmeterol and fluticasone in managing COPD exacerbations in patients with a history of exacerbation in the previous year.

Source: www.nejm.org

Liraglutide Added to High-Dose Insulin Therapy Improved Glycaemic Control

Date: June 6, 2016

Although high-dose insulin therapy is increasingly common for treating patients with type 2 diabetes, it is associated with weight gain, hypoglycaemia and high treatment burden. Liraglutide, a glucagon-like peptide 1 receptor agonist, is a candidate for combination therapy due to its ability to induce weight loss, increase glucose-dependent insulin release and decrease glucagon secretion.

A double-blind, placebo-controlled, randomized and balanced study has investigated the effectiveness and safety of combined regimen of liraglutide and high-dose insulin in patients with type 2 diabetes. From 2012 to 2015, 71 patients were recruited from ambulatory clinics at the University of Texas Southwestern Medical Center and Parkland Hospital. They all had type 2 diabetes (HbA1c 7.5% - 11.0%) requiring high-dose insulin (> 1.5U/kg/d). During the 6-month follow–up, patients were divided into two groups (insulin with liraglutide or with placebo). HbA1c level was the primary outcome while change in weight, hypoglycaemia rate, insulin dosage and quality-of-life were evaluated as secondary outcomes.

Liraglutide added to high-dose insulin therapy improved glycaemic control (treatment difference 0.9%, P = 0.002), decreased body weight (-2.3 kg, P = 0.02), and enhanced treatment satisfaction. There was no significant difference in the rates of hypoglycaemia and other adverse events.

This research agrees with similar studies regarding the benefits of liraglutide to patients with type 2 diabetes requiring high-dose insulin. More studies are needed to investigate the long-term effect of the combination therapy.

Source: jama.jamanetwork.com
Canagliflozin: Signal of Increased Risk of Lower Extremity Amputations Observed in Trial in High Cardiovascular Risk Patients

Date: June 16, 2016

The Medicines and Healthcare products Regulatory Agency (MHRA) advised that a signal of increased lower limb amputation (primarily of the toe) in people taking canagliflozin compared with placebo in a clinical trial in high cardiovascular risk patients is currently under investigation.

Canagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor indicated in adults with type 2 diabetes mellitus to improve glycaemic control when diet and exercise alone do not provide adequate glycaemic control. Canagliflozin is given as monotherapy in patients for whom the use of metformin is considered inappropriate due to intolerance or contraindications. Canagliflozin can also be given as add-on therapy with other glucose-lowering drugs, including insulin, when these do not provide adequate glycaemic control.

The canagliflozin-containing medicines marketed in the UK are Invokana ▼ (canagliflozin) and Vokanamet▼ (canagliflozin and metformin).

CANVAS trial

The incidence of lower limb amputation (primarily of the toe) is higher in the canagliflozin groups compared with the placebo group in a clinical trial of high cardiovascular risk patients (CANVAS, an on-going long-term cardiovascular outcomes trial). The trial is fully enrolled with 4,330 randomised participants. The mean and median follow-up time is approximately 4.5 years.

The incidence of lower limb amputation is 7 per 1000 patient-years in the canagliflozin 100 mg group and 5 per 1000 patient-years in the canagliflozin 300 mg group, compared with 3 per 1000 patient-years in the placebo group.

This increased risk was observed independent of risk factors. However, the absolute risk was higher in patients with previous amputations, existing peripheral vascular disease, or neuropathy. No dose response was observed.

Any possible mechanism behind these events is as yet unknown. However, dehydration and volume depletion might increase this risk. The MHRA therefore recommends that healthcare professionals to follow the interim advice outlined below while this signal is being investigated by the European Medicines Agency. The results of the review will be communicated when available.

Other trials: no significantly increased risk observed

In an ongoing outcome trial with a similar population to CANVAS, the CANVAS-R trial, there have been 16 amputations in the canagliflozin group and 12 amputations in the placebo group. The estimated annualised incidence of amputations is 7 per 1000 patient-years in the canagliflozin group compared with 5 per 1000 patient-years in the placebo group (no statistically significant difference).

- 12 completed phase 3 or 4 trials have shown no increase in amputation incidence with canagliflozin (incidence of 0.6 per 1000 patient-years in canagliflozin groups and 2 per 1000 patient-years in control groups; mean follow-up of 0.9 years).

Advice for healthcare professionals:

- As a precaution, consider stopping canagliflozin if a patient develops a significant lower limb complication (eg, skin ulcer, osteomyelitis, or gangrene), at least until the condition has resolved, and continue to monitor the patient closely.
- Carefully monitor patients receiving canagliflozin who have risk factors for amputation (eg, previous amputations, existing peripheral vascular disease, or neuropathy).
- Monitor all patients for signs and symptoms of water or salt loss; ensure patients stay sufficiently hydrated to prevent volume depletion; note that diuretics can exacerbate dehydration.
- Advise patients to:
  - stay well hydrated
  - carry out routine preventive foot care
  - seek medical advice promptly if they develop skin ulceration, discoloration, or new pain or tenderness
  - start treatment for foot problems (eg, ulceration, infection, or new pain or tenderness) as early as possible
  - continue to follow standard treatment guidelines for routine preventive foot care for people with diabetes.


New Medicine Labelling Requirements to Take Effect from August 5, 2016

Date: July 25, 2016

The Department of Health (DH) reminded the pharmaceutical trade and the public that provisions under the Pharmacy and Poisons Ordinance (Cap 138) (PPO) in relation to the labelling of pharmaceutical products containing poisons will take effect on August 5, 2016 to alleviate unnecessary concerns of consumers that such products might be harmful and unsuitable for use or consumption.

According to section 27 of the PPO, no person shall sell any poison unless the container is labelled in accordance with the Pharmacy and Poisons Regulations (Cap 138A) (PPR). Under the PPR, the container of a poison-containing pharmaceutical product must be clearly printed with both English and Chinese text as specified in Schedule 5 in respect of the kind of poison contained. The text must not be modified in meaning by the addition of any other text or marks.

Depending on the sale restriction, the new labelling requirements are:

For medicine containing a poison included in Schedule 3 of the PPR, it must be labelled with the text “Prescription Drug 處方藥物”; and
Implementation Plan of Phase 2 Requirement of Bioavailability and Bioequivalence Studies for the Registration of Generic Drugs

Date: June 2016

In 2009, the Review Committee on the Regulation of Pharmaceutical Products in Hong Kong recommended to require bioavailability and bioequivalence (BABE) studies as registration requirement for generic drugs and to implement the requirement by phases. In April 2010, the Pharmacy and Poisons (Registration of Pharmaceutical Products and Substances: Certification of Clinical Trial/Medicinal Test) Committee (the Registration Committee) implemented the Phase 1 requirement for BABE studies covering 29 antiepileptic drugs.

In August 2013, the Pharmacy and Poisons Board endorsed the decision to establish an Expert Advisory Group on BABE studies (the BABE Expert Group) to facilitate the above implementation. The BABE Expert Group was chaired by the Assistant Director (Drug) and included 11 local members from the academia, pharmaceutical trade, Hospital Authority, Department of Health, medical and pharmacy professions, and 2 overseas co-opted members. In March 2016, the BABE Expert Group endorsed an implementation plan and timeline on the Phase 2 requirement for BABE studies for consideration by the Registration Committee. In June 2016, the Registration Committee decided to implement the Phase 2 requirement for BABE studies covering 38 Critical Dose Drugs / Narrow Therapeutic Range Drugs (NTRD) at Annex according to the following requirements and timeline:

Requirements:

Applications of the 38 Critical Dose Drugs / NTRD at Annex must include BABE studies in accordance with the World Health Organization (WHO) guidance document - Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Other BABE studies can be accepted if official evidence of registration approval of the generic drugs in the following countries or region can be provided: Australia, Canada, the European Union, Japan or the United States.

Timeline:

A. New applications for registration received before 1 August 2016

For all the new applications for registration of the 38 Critical Dose Drugs / NTRD at Annex received before 1 August 2016 but have not been completed for registration before 1 August 2017, the applicants must satisfy the Phase 2 requirement for BABE studies before the approval of the applications.

B. New applications for registration received on or after 1 August 2016

With effect from 1 August 2016, all the new applications for registration of the 38 Critical Dose Drugs / NTRD at Annex must include evidence to satisfy the Phase 2 requirement for BABE studies. Otherwise, the applications will not be accepted for evaluation.

C. Renewal applications of registered generic drugs

With effect from 1 August 2017, all the renewal applications for registration of the 38 Critical Dose Drugs / NTRD at Annex must include evidence to satisfy the Phase 2 requirement for BABE studies. Otherwise, the registration of the generic drugs will not be renewed.

Annex 38 Critical Dose Drugs / Narrow Therapeutic Range Drugs:

Acetohexamide
Aminophylline
Aprindine
Chloramphenicol
Choline theophylline
Clindamycin
Clonidine
Cyclosporine
Digitoxin
Digoxin
Diprophylline
Disopyramide
Ethinyl Estradiol
Flecainide
Glibenclamide
Gliclazide
Glybuzole
Glyclopyramide
Guanethidine

Isoetharine
Isoprenaline
Levodopa and Carbidopa
Levothyroxine
Lithium
Metaproterenol
Methotrexate
Minoxidil
Phenobarbital
Prazosin
Procalamine
Proxphylline
Quinidine
Sirolinus
Tacirolimus
Theophylline
Tolazamide
Tolbutamide
Warfarin

A Lecture Review on “Coaching – Practical Exercises, Discussion and Case Examples” by Charlie Lang

(Hong Kong Pharmacy Conference 2016 Concurrent Session III)

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ABSTRACT

Amongst the concurrent sessions in The Hong Kong Pharmacy Conference 2016, the two-part practical lecture presented by Charlie Lang (Figure 1) appeared to be a unique one - in terms of both content and teaching style. While the informative knowledge-sharing seminars drew much attention of the delegates, it was definitely worthwhile to hear Charlie’s insightful introduction on Coaching and to apply the corresponding soft skills in the pharmacy-based practical workshop.

The lecture opened with an overview of Charlie’s innovative approaches towards leadership and coaching. As the founder and managing partner of Progress-U Asia, Charlie has accumulated over 4000 hours of executive coaching experience, underlining his passion and commitment, which the audience could catch a glimpse of, in the following few hours of lecture.

WHAT IS COACHING?

Charlie described that, Coaching is a set of communication skills assisting employers to manage their employees, with the aim of increasing their engagement in the workplace. As defined by Charlie, engagement is usually expressed in three ways, or for the ease of memorization – 3S. “Say”, when employees consistently speak positively about the organization to co-workers, potential employees and customers; “Stay”, when employees have an intense desire to be a member of the organization; “Strive”, when employees exert extra effort (also known as Discretionary Effort) and engage in behaviors that contribute to business success. It is not difficult to agree with Charlie’s statement that, through the Coaching process, employees are encouraged to be more engaged in their work, facilitated to put extra time and effort into their career, and eventually they benefit the company by earning more profit and market value.

However, how can one relates coaching to the pharmacy profession, for which monetary incentives are usually not the ultimate goal?

WHY COACHING?

It was clarified by Charlie’s illustration, in a “Healthcare Setting”, Coaching resembles the job of healthcare professionals: Diagnosis should be made, to identify the root cause of the employee’s problem, and subsequently treatment targeting the root cause can be given. Pharmacists can be leaders in different business and usually bear the roles of managers, supervisors or even employers. Coaching helps pharmacists offer guidance on problem-solving and goal-setting and stimulate staffs’ reflective thinking and personal development. Therefore, mastering individual’s coaching skills could be very meaningful and rewarding for both the coacher and the coachees.

HOW TO BE A COACHING PHARMACIST?

Charlie used a straightforward and comprehensive framework (Figure 2) as a tool to demonstrate the application of the soft skills.

Charlie proposed that everybody can be a coaching pharmacist by possessing the followings:
I. An Optimal Mindset

According to Charlie, an optimal mindset consisted of “giving ownership”, “being non-judgmental” and “forward ownership”, which are essential elements for a coacher to carry out an impactful coaching.

“Giving Ownership” makes Coaching stands out among other communication approaches, for example counseling and consultation. The coacher has to bear in mind that, no advices should be given to the coachee, who is the only person in control of the conversation. In other words, guiding questions like “What do you want to achieve?” or “How would you achieve it?” are preferred to sentences like “I think you should practice more.” or “Maybe you should set the deadline on next Friday.”

“Being Non-Judgmental” is another key factor in the coaching mindset. Undoubtedly, no one would enjoy the conversation if his/her decisions or statements are criticized. Being judgmental will push the coachee towards a defensive stance, and refuses to express more.

“Forward Orientation” is the aim of Coaching. The conversation should be away from past failures and focus on strategic future planning, for the sake of turning problems into opportunities for potential achievements.

With an optimal mindset, the coacher sets the scene of openness to allow the coachee to express himself freely, which is fundamental for starting a meaningful coaching session.

II. Essential Coaching Competencies

The coacher should also be equipped with essential coaching competencies. The first step is to “Build Trust” with the coachee under optimal care, rapport and competence. Throughout the conversation, the coacher needs to be intensively involved, even though the conversation ownership is not in his/her hands. “Being Fully Present”, “Active Listening”, “Powerful Questioning” and “Giving Feedback & Direction Communication” are the keys for being attentive, as stated briefly by Charlie.

By accomplishing these, the coacher can maintain a good relationship with people involved in the conversation, hence boost the coachee's willingness to open up and express himself/herself proactively.

III. The GROW Model

Much attention was paid by Charlie to the GROW Model, to which the coacher have to adhere throughout the conversation process, with the intention of guiding a general direction for the coachee to achieve a meaningful and efficient communication.

“G” stands for “Goal”. Questions are asked to encourage the coachee to set his/her own goal. It can be the goal of this conversation, or even the goal of a future plan. For example, “What would you like to get from this conversation?” or “What would you like to achieve by solving this problem?”

“R” stands for “Reality”. The coachee is stimulated to reflect on current situation and discover the root cause of ongoing issues. For example, “What is the problem you are facing now? What have you done in response to it?”

“O” stands for “Options”. The coacher has to assist the coachee in brainstorming solutions for the concerning matter. For example, “What can you do to deal with this problem? Is there any opportunity lied in this problem?”

“W” stands for “Way Forward”. As stated in the Coaching Mindset, Coaching is always forward-oriented. At the end of conversation, the coachee has to set future target regarding the current situation. For example, “What are you going to do now? How confident are you?”

By following the GROW Model, everyone has the potential to manage a coaching conversation. However, practice makes perfect. As shared by Charlie, only years of experiences and numerous case studies can shape one into a successful coacher.

CASE STUDY

A coaching conversation between a senior pharmacist and a dispenser with poor workplace performance. With Charlie acting as the senior pharmacist (the coacher) and the course moderator being the dispenser (the coachee), three different approaches of communication (Figure 3) were demonstrated:
I. Directing

In the first round of conversation, direct instruction was given to the dispenser, ordering him to figure out his own problem and perform better. The one-way communication was clearly not pleasant, for both the coachee and the audience. No doubt this would be the most straightforward way of staff management, yet this might not be the perfect way, in terms of engagement building.

II. Consulting

The second round of conversation was generally advice-giving. While coacher talked to the coachee and tried to delve into the origin of current issue, sympathy was shown, and valuable advices were given. This approach of connection was way more amiable than the previous one, but was undeniably only attainable when the coacher is sophisticated enough for giving decent recommendations.

III. Coaching

The last round of conversation was a two-way communication, in which the coachee participated more proactively than the previous cases. “GROW” Model was implemented, the coachee revealed his underlying family issue and set personal goals to achieve future improvement and development.

After the demonstration of the use of the previous two styles, it was agreed that Coaching was the best among the three approaches illustrated. The non-directive discussion was efficient and eventually resulted in a forward-looking conclusion.

By observing Charlie’s role play and personally took part in a few more case studies, the advantages of Coaching was conspicuous. Unlike an instructive or directive discussion, Coaching is a communication with the nature of inspire. In another word, the coacher does not need to be an expert to give advice, but is required to guide and stimulate reflective thinking of the coachee instead, targeting specific problem and focusing on improvement. Anybody can handle it, but of course, after thorough training and practicing.

REFLECTION

In this introduction lecture on Coaching, the brilliant side of this communication skill was clearly exhibited. However, the imperfect side of Coaching can be found under judicious consideration. A communication involves two parties, and in the case of Coaching, the coacher and the coachee. Following the guidance of Charlie, we could say the coacher has to be equipped with abundant techniques and experiences to carry on a successful coaching discussion as mentioned above.

On the other hand, a coachee should have self-awareness, so that root cause of the problem can be addressed. Together with adequate motivation and capability to improve, individual goals can be set and eventually targets can be met independently.

As someone who has never heard of Coaching, this lecture provided a number of new insights for me. I cannot agree more with Charlie, that efficient communication is critical for staff management and career development, in either business world or healthcare system. In the pharmacy profession, Coaching is a soft skill that everybody should possess. I hope Charlie’s lecture in this year’s Pharmacy Conference can draw some attentions from both senior and junior members in the pharmacy profession and popularize this favoring communication skill among everyone.
An Overview of the Pharmaceutical Properties of Oral Care Products

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ABSTRACT
The most recent citywide oral health survey is reviewed, which suggested the oral health status in Hong Kong is suboptimal. Not only oral conditions persist, oral care knowledge is also insufficient in majority of the public. Oral care could best be provided by dentists, however the utilization of professional dental care has not been extensive. Pharmacists are conveniently accessible to citizens with counseling service free of charge, they could take a unique advantageous position in providing basic oral care education and referring patients at need to dentists. This article provides an overview of the typical oral care products available in community pharmacies. Basic pathophysiology of common oral conditions, pharmaceutical properties and precautions of oral care products are discussed. It intends to enhance pharmacists’ basic understanding related to oral care, and encourage them to take an active role to contribute to the general oral health status of the Hong Kong population.

Keywords: oral health, toothbrushes, toothpastes, mouthwashes

INTRODUCTION
Oral health is integral to general health and good quality of life.(1) It is characterized by the absence of conditions that affect the oral cavity, such as mouth and tooth pain, oral sores, periodontal disease, tooth decay and tooth loss. Worldwide, almost 100% of adults have dental cavities, while up to 60-90% of school children are also affected.(1)

In Hong Kong, the Department of Health conducts oral health survey every 10 years to assess oral health status in the community and to provide relevant information for healthcare professionals to improve the oral health of local people. The latest survey was conducted in 2011 on five selected age groups: people who were 5, 12, 35-44 years old, non-institutionalized elderly aged 65-74 and elderly user of Long-Term Care Services aged 65 or above. It was revealed that various degrees of tooth decay and gum diseases persisted among all selected age groups. Majority of the population did not have regular dental check-up, and tended to delay seeking dental care until the symptoms cause disturbance to sleep. In terms of knowledge and oral care habits, most of the respondents could not relate the harm of smoking, frequent eating and drinking, and lack of regular dental check-up to oral health. Knowledge on the use and benefit of fluoride toothpaste was far from satisfactory. Full rate of twice-daily brushing and daily flossing habit was not well-established. There was misbelief that oral self-care could replace professional dental care. The situation in elderly deserves even more concerns. Gum inflammation, bleeding and gum pocket was prevalent and extensive. Their oral hygiene practice was ineffective in maintaining oral health, basic technique of oral care should not be presumed. Moreover, their utilization of oral healthcare service was low, which was related to incorrect perception of self-recovery and financial concerns.(2)

The local survey has suggested several measures to improve oral health. Routine oral care with tooth brushing, flossing and appropriate use of fluoride should be reinforced. Different disciplines are encouraged to work together to develop a culture that values the significance of oral health in overall well-being, through education on prevention and early treatment of dental diseases.(2)

By virtue of their frequent contact with citizens, it is acknowledged that pharmacists could actively participate in oral healthcare.(3) Pharmacists’ service are conveniently accessible and are free-of-charge, enabling them to be the first point of professional contact when dental issues are encountered. In Hong Kong, varieties of oral care products are widely available in community pharmacies. Pharmacists can play an active role in assisting citizens to select suitable products to maintain oral hygiene. Pointed out by the oral health survey, there is a gap between the public’s dental needs and the utilization of professional dental care. This article aims to equip pharmacists with essential knowledge on oral health by introducing the basic pathophysiology of common dental issues and the functions of typical oral care products. Educating the public on oral healthcare principles with comprehensive product counseling, and timely referral to dentist upon identification of oral health problems, pharmacists could bridge the gap.

ORAL CARE PRODUCTS
Oral care products available in pharmacy are mainly toothbrushes, dental floss, toothpastes and mouthwashes. Each of them has essential function in maintaining oral hygiene. Understanding the causes of common dental diseases, and the properties of different products and their ingredients enables wise selection of oral care products for individuals with different needs.

Toothbrushes and dental floss
Dental plaque is a thin film of bacteria adhering on the surface of teeth. It accumulates along the gingival margin, releases toxins that irritate the periodontal tissues causing gum inflammation. In severe periodontitis with prolonged inflammation and destruction, gum and supporting bone
recede leading to mobile and drifted teeth or eventually tooth loss.\(^{2,4}\) The function of toothbrushes and dental floss is to remove dental plaque from tooth surfaces. Both traditional manual toothbrushes and powered toothbrushes clean the teeth effectively as long as they are used correctly, while dental floss enhances the cleansing of adjacent surfaces.\(^{4}\)

Manual toothbrushes vary in bristle pattern, size, shape and handle design. Product selection should be individualized. Firstly, the size of the toothbrush head should be appropriate to the size of the oral cavity to enable itself to be maneuvered effectively inside the mouth to clean every tooth surface. To illustrate, the size of the toothbrush head should be approximately 15mm for children aged 0 to 2 years old; and approximately 25mm for adults in general.\(^{4}\) The ability to remove plaque would be compromised and may cause gagging if the head size is too large, whereas excessive time may be needed for brushing if it is too small.\(^{5}\) Secondly, the bristle should be soft and of round ended filaments in compact arrangement in order to reduce injuries to the gingival tissues and cervical margins of the teeth.\(^{5,6}\) Synthetic materials of nylon texture are preferred as they are less porous and hence less likely to harbor bacteria in the bristles.\(^{5}\) Thirdly, the handle should be of appropriate length and thickness for comfortable and firm grip.\(^{6}\) Brush head design with various bristle pattern can be chosen according to personal preference as long as brushing is performed with correct techniques.\(^{4}\)

On the other hand, powered toothbrushes are differentiated by the mechanical motions of the toothbrush head, which include unidirectional, rotational and ultrasonic vibration.\(^{4}\) Although manual toothbrushes with proper brushing techniques are just as good as powered toothbrushes, it was found that powered toothbrushes with rotation oscillation action reduce plaques and gingivitis more effectively.\(^{2,7}\) Since the technique for using powered toothbrushes is different from that of manual toothbrushes, users should read the instructions in the package insert thoroughly, or consult their dentists to achieve the best cleansing effect.\(^{4,5}\) No matter which type of toothbrushes is being used, replacement of the toothbrush head should be performed every 3-4 months, or when signs of wear such as splaying of bristles appears.\(^{9}\)

Dental floss is composed of nylon filaments bounded together to form a thread. It is used to clean the adjacent tooth surfaces with sawing motion in the interdental space. There are flattened, round and spongy floss on the market, any kind will effectively remove plaque when used correctly.\(^{4}\)

**Toothpastes**

Toothpastes are used in conjunction with a toothbrush to maintain oral hygiene. The main purpose is to help remove debris and dental plaque. Fluoride toothpaste, anti-plaque toothpaste, anti-calcification toothpaste, desensitizing toothpaste and whitening toothpaste are common types of therapeutic toothpastes. Their therapeutic effects are dependent on the active ingredients contained.\(^{4}\)

Excluding the unique therapeutic agents, most toothpastes contain common constituents. For teeth cleansing, abrasives, e.g. calcium and sodium salts (calcium carbonate, calcium sulphate, dicalcium phosphates, sodium bicarbonate) and silica particles, are present to enhance the scrubbing action to clean and polish the tooth surfaces. Foaming agents, such as sodium lauryl sulphate or sodium N-lauryl sarcosinate, reduce the surface tensions and facilitate the removal of debris. For structural support and stability of the toothpaste, humectants, such as glycerine and sorbitol, serve to prevent water loss and thus keep the toothpaste moist. Binding agents, like natural gums and synthetic cellulose, stabilize the toothpaste formula, increase viscosity and provide the desirable creamy texture. Sodium benzoate, formalin and alcohols are commonly used preservatives to prevent contamination by bacteria. Flavoring agents, e.g. peppermint, menthol and eucalyptus, promote user compliance by giving a sense of well-being with a pleasant tasting mouth and fresh breath.\(^{2,5}\)

Dental cavities can be prevented by maintaining a constant low level of fluoride in the oral cavity.\(^{1}\) Tooth decay is the result of an imbalance between demineralization and remineralization of the enamel, often due to frequent eating and drinking.\(^{10}\) The prevalence has declined in the past decades since the introduction of fluoridated toothpastes, which contains sodium fluoride, sodium monofluorophosphate or stannous fluoride.\(^{4,5}\) Demineralization occurs when the enamel is dissolved by acid produced by oral bacteria in the metabolism of food remnants and debris.\(^{10}\) Fluoride enhances remineralization and repairs the partly dissolved enamel crystallites in early stage of cavities. When incorporated into the crystallite structure as fluorapatite, fluoride increases the resistance to further acid attack and inhibits demineralization. It also interferes the formation and functioning of dental plaque micro-organisms.\(^{10,11}\) In short, fluoride repairs damaged teeth and decreases the vulnerability to further damage. Toothpaste with a fluoride content of 1000 parts per million (ppm) or above is considered effective in preventing tooth decay. Children may use toothpaste with lower fluoride concentration 500ppm with parental supervision on brushing to prevent excessive ingestion of fluoride.\(^{4}\)

Anti-plaque toothpastes target on the accumulation of biofilm of bacteria adhered on the tooth surfaces. Triclosan and zinc citrate are ingredients commonly used for their antibacterial property.\(^{5,12}\) Triclosan induces membrane leakage and hence cause lysis of bacteria, whereas zinc inhibits microbial glucose uptake and metabolism.\(^{12}\) Anti-plaque toothpastes inhibit plaque accumulation, reduce the exposure of gum tissues to bacterial toxins and thereby reduce the risk of developing gum diseases and dental cavities.\(^{4}\) Dental plaque can be calcified by saliva to form calculus, of which the rough surface can further trap more dental plaque.\(^{2,4}\) Anti-calcification toothpastes, with active ingredient of pyrophosphate or zinc citrate, delay plaque mineralization by inhibiting the growth of calcium crystals.\(^{5,12}\) Nevertheless, effective removal of plaques and calculus cannot solely rely on therapeutic toothpastes. Mechanical actions like proper tooth-brushing and professional dental cleansing are of paramount importance.

Teeth become yellowish or brownish when stains accumulate with habitual smoking or consumption of dark-coloured beverages such as coffee and tea.\(^{4}\) Whitening toothpastes mainly contain detergents and coarse abrasives, e.g. silica and sodium tripolyphosphate, which abrade the extrinsic stains on tooth surface.\(^{5,12}\) It should be noted that excessive abrasion may damage tooth enamel and cause tooth sensitivity. Hence, manufacturer’s recommendation on proper use of whitening toothpaste products should be followed carefully.\(^{16}\) Some whitening toothpastes achieve the whitening effect optically. The active ingredient blue covarine deposits on the surface of the teeth and creates an optical illusion through colour shift that make the teeth appear less yellow.\(^{13}\) Peroxide contained in other professional tooth-whitening products oxidizes the organic matrices in enamel to lighten the
intrinsic tooth colour. It should be used under the supervision of a dentist as improper use may injure the gum and tooth-supporting tissues.\(^\text{4,14}\)

Dentin hypersensitivity is a common complaint among citizens. It is characterized by sudden, sharp pain when consuming hot, cold, acidic or sugary food and beverages, or upon physical contact with a toothbrush or dental floss.\(^\text{15,16,17}\) The dentin, normally covered by enamel or cementum, has tubular structure connecting to the pulp which contains nerves. When dentin is exposed as a result of acid erosion, abrasion, abrasion, gingival recession or periodontal diseases, external stimuli trigger fluid movement in dentinal tubules which transmits the stimuli to the nerve endings in the pulp causing transient pain.\(^\text{16,17,18}\) Desensitizing toothpastes relieve dentin hypersensitivity by occluding the dentinal tubules or interrupting the neuronal response to pain stimuli.\(^\text{16}\) Arginine and calcium carbonate are occluding agents commonly found in toothpastes. They form a protective layer and seal the dentinal tubules to reduce dentin fluid flow.\(^\text{12,18}\) On the other hand, potassium nitrate exerts the nerve calming effect by building up potassium ions in the dentin fluid, where a depolarizing effect on nerve conduction is achieved, leading to a reduction in nerve fiber excitability. Most potassium-based desensitizing toothpastes require continued use over a 4- to 8-week period to show sustainable pain relief.\(^\text{18}\)

### Mouthwashes

Mouthwashes are aqueous solution used primarily for deodorant or antiseptic effect.\(^\text{12}\) They are formulated to help remove debris, reduce oral bacterial count and provide a pleasant after-taste. Although they cannot replace brushing and flossing in daily dental care, one might consider incorporating mouthwash as adjunct as it may offer additional benefits in terms of plaque removal and prevention of gingivitis.\(^\text{4}\)

Mouthwashes are divided into cosmetic and therapeutic mouthwashes. Cosmetic mouthwashes temporarily reduce bad breath by leaving a pleasant after-taste and remove debris by gargling action. They are not effective in reducing plaque, gingivitis and cavities.\(^\text{4,14}\) Therapeutic mouthwashes, marketed for anti-plaque, anti-decay or desensitizing function, contain active ingredients similar to those found in toothpastes. Triclosan, essential oils (e.g. thyme, menthol and eucalyptol), chlorhexidine and cetylpyridinium chloride are often included in anti-plaque mouthwashes for their antibacterial activity.\(^\text{4,5,12}\) Chlorhexidine is one of the most effective antiseptic agents for mouthwash formulation with broad spectrum activity against gram-positive bacteria, gram-negative bacteria, anaerobes and yeast. It is bacteriostatic at low concentration, interfering bacterial membrane permeability and transport; and bactericidal by precipitating bacterial cytoplasmic content at high concentration.\(^\text{12,20}\) It also disrupts the structure of existing dental plaque, hence prevent bacteria and salivary glycoprotein from further binding.\(^\text{21}\) However, discoloration of oral surfaces with brownish stain may occur as a side effect.\(^\text{22}\) As for cetylpyridinium chloride, it possesses surfactant property, which can disrupt bacterial membrane integrity, subsequently induce membrane leakage and cell death.\(^\text{23}\) Supplementing mechanical cleaning with anti-plaque mouthwashes enhance bacterial killing and reduce gum inflammation. Other therapeutic mouthwashes include mouthwashes with extra fluoride compounds providing additional protection against tooth decay, and products containing arginine against tooth hypersensitivity.\(^\text{14}\) For the purpose of freshening breath, antibacterial mouthwashes may help by eradicating the odour-producing bacteria in the mouth. Nevertheless, good oral hygiene should be maintained with day-to-day oral care. If no improvement is seen despite maintenance of oral hygiene, dental or medical advice should be sought, as persistent bad breath could be signs of infection or inflammation in the nose, sinuses, mouth or throat.\(^\text{24}\) Public should also be reminded that therapeutic mouthwashes should never replace brushing and flossing, except under certain circumstance as instructed by dentists, for instance, post oral surgery where tooth-brushing may be hindered.\(^\text{4}\)

Some mouthwashes contain a significant amount of alcohol. Alcohol has antibacterial effect, and serves as a carrier for flavoring agents.\(^\text{25}\) However, its drying property may aggregate the condition in patients with xerostomia, e.g. patients on anticholinergic medicines, receiving radiotherapy to the head and neck region, or have underlying medical conditions such as Sjögren’s syndrome.\(^\text{26}\) Dry mouth may also worsen bad breath as saliva and its enzymes are important to remove dead cells or particles that may cause odour.\(^\text{24}\) Many commercial brands are now marketing alcohol-free mouthwashes to avoid the side effect of dry mouth.

### Health Issues and Concerns

Oral care products are effective tools to maintain oral hygiene, however they are not completely free from health concerns.

Alcohol consumption is a known risk factor for oropharyngeal cancer. As some alcohol-containing mouthwashes contain up to 20% volume of ethanol, possible carcinogenicity from mouthwashing has been considered. In 2007, an international, multicentre, case-control study reported an odd ratio of 3.4 for the risk of developing oral cancer in individuals who used mouthwashes daily, independent of tobacco and alcohol consumption.\(^\text{28}\) It was proposed that the extra-hepatic metabolism of alcohol to mutagenic acetaldehyde in the oral cavity and the enhanced mucosal penetration result in carcinogenic effect. There has been proposition to avoid using alcoholic mouthwashes for long term.\(^\text{27}\) However, various epidemiology studies conducted over the past few decades showed inconsistent results about the linkage of mouthwash and oropharyngeal cancer.\(^\text{28,29,30}\) A science brief issued by the American Dental Association in 2009 did not support the connection.\(^\text{31}\) No association was reported in a recent meta-analysis.\(^\text{32}\) Although available evidence does not support a causal relationship between alcoholic mouthwashes and the development of oropharyngeal cancer, any potential risk shall be assessed with more well-designed research studies. Concerned customers or individuals who are at higher risk of developing oral cancer due to lifestyle or genetic factors may consider using alcohol-free mouthwashes for the maintenance of oral health. It may be prudent to advise individuals who smoke or have aldehyde dehydrogenase deficiency to limit the use of alcohol-containing mouthwashes.\(^\text{31}\)

The antibacterial chemical, triclosan, commonly found in toothpastes and mouthwashes is under evaluation by the U.S. Food and Drug Administration(FDA). It is not currently known to be hazardous to human, but studies suggested that it might alter hormone regulation in animals, and promote the development of antibiotic resistance in bacteria. At present, FDA acknowledges triclosan-containing products effective in preventing gingivitis and there is insufficient safety evidence to recommend change in its usage.\(^\text{33}\) Nevertheless, FDA has been reviewing the safety and effectiveness of triclosan in hand soaps and body washes, and may incorporate more information in their regulations that govern the use of the chemicals in the future.
Young children should use oral healthcare products under parental supervision, especially when using products that contain fluoride. Excessive ingestion of fluoride can result in fluorosis, which presents as white patches and surface irregularities on the enamel surface. It can be cosmetically disfiguring when fluoride over-exposure occurs during enamel formation of the permanent upper anterior teeth during childhood. Furthermore, children under the age of 6 are not recommended to use mouthwashes in general, for the reason that they have yet to acquire the skills to swish and spit leading to potential risk of accidental ingestion. It is important to make sure children are able to swish with a sip of water before trying mouthwashing.

CONCLUSION

Oral health means more than good teeth. It is vital to an individual's capacity in biting, chewing, smiling, speaking and psychosocial well-being. The general oral health status in Hong Kong is below optimal. Not only various degrees of tooth decay and gum diseases persist, knowledge on oral care is also inadequate. As an indispensable member of primary healthcare, pharmacists are advised to equip themselves with relevant knowledge in oral health, and the pharmaceutical properties of typical oral care products, in order to handle common oral care issues of the general public and to actively contribute to improve oral health of the local population.

Author’s background

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References


Questions for Pharmacy Central Continuing Education Committee Program

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

1. Which of the following statements regarding dental plaque is incorrect?
   A. Dental plaque is a thin film of bacteria and their products adhering on the tooth surfaces.
   B. Calculus is dental plaque which is hardened as a result of deposition of minerals from saliva.
   C. Dental plaque releases toxins which irritate the gum tissue leading to gum inflammation.
   D. Gargling with chlorhexidine mouthwash is more effective than brushing in plaque removal.

2. Dental cavities can be prevented by
   A. Twice daily tooth-brushing with fluoride toothpaste
   B. Avoiding snacking in between regular meals
   C. Having regular dental check-up
   D. All of the above

3. Which of the following statements regarding the selection of toothbrush is incorrect?
   A. The toothbrush head should be approximately 25mm for adults.
   B. Bristles of natural materials are preferred to reduce mechanical injury to the gingival tissues.
   C. The handle should be of appropriate length and thickness for comfortable and firm grip.
   D. Replacement of the toothbrush head should be performed every 3-4 months.

4. Which type of motions of powered toothbrush has the greatest evidence in reduction of plaque?
   A. Unidirectional vibration
   B. Ultrasonic vibration
   C. Rotation oscillation
   D. Sawing motion

5. What is the suitable fluoride content in toothpaste for adults?
   A. 100ppm
   B. 500ppm
   C. 800ppm
   D. 1000ppm

6. Which of the following statements regarding mouthwash is incorrect?
   A. Therapeutic mouthwash should not replace brushing and flossing.
   B. Young children should use mouthwash for better protection of their immature teeth.
   C. Alcohol content in mouthwash may aggregate dry mouth.
   D. Alcohol-free mouthwash is preferred for individuals who are at high risk of developing oral cancer.

7. Which of the following is the mechanism of arginine to relieve dentin hypersensitivity?
   A. Occlude dentinal tubules
   B. Enhance remineralization of enamel
   C. Reduce nerve fiber excitability
   D. All of the above

8. Which of the following practice to reduce dentin hypersensitivity is not true?
   A. Use toothbrush with soft bristles
   B. Avoid biting bones and nuts
   C. Use toothpaste containing potassium nitrate
   D. Use toothpaste containing blue covarine

9. Excessive ingestion of fluoride can lead to
   A. Worsened bad breath
   B. Altered development of the enamel of teeth
   C. Development of antibiotic resistance in oral bacteria
   D. Discoloration of oral surfaces with brownish stains

10. Pharmacists can contribute to the oral health of the population by
    A. Aiding the selection of suitable oral care products for individuals with different needs
    B. Providing basic education on oral health principles (e.g. smoking cessation, establishing good dietary habit)
    C. Referring patients at need to dentist to avoid delay in dental treatment
    D. All of the above

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**Biogenesis Regulation and Detection Methods of MicroRNA Expression Profiling**

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**ABSTRACT**

Human genome contains more than 97% non-coding nucleotides. It is now recognized that numerous intronic or intergenic regions of mammalian genome encode various microRNAs (miRNAs), which are responsible for RNA-mediated gene silencing through RNA interference (RNAi)-like pathways. Particular expression profiles of miRNAs have been found to associate with some particular pathogenesis. Hence, establishing expression profiles of miRNAs has become an important means for prognosis and diagnosis of human diseases, including cancer as well as for the study of gene regulation. Traditional methods adopted for miRNA detection have various limitations. But innovative methods have been developed for increased sensitivity and specificity of miRNA detections and quantifications. This review focuses on the basic concepts of miRNA biogenesis and summarizes miRNA detection methods focusing more on the newly developed methods.

**Keywords:** miRNAs, canonical & non-canonical miRNA biogenesis, expression profile, qRT PCR, miRNA microarray, RNA Seq.

**INTRODUCTION**

MicroRNAs (miRNAs) are short endogenous, noncoding single-stranded RNA molecules of 20-22 nucleotide that control the expression of mRNAs in a sequence specific manner associated with numerous biological processes. Therefore, deregulated miRNAs play a significant role in the pathogenesis of diseases. miRNAs have emerged as the crucial players in gene expression regulation, and involved in cell differentiation, proliferation, and apoptosis and are implicated in many types of diseases. miRNA expression profiles differ between healthy and diseased tissue. Moreover, miRNAs circulate in blood in unexpectedly high stable form. Thus, circulating miRNAs or tissue–based mRNA have the remarkable potential to be developed as diagnostic, prognostic, and predictive biomarkers of several human diseases including, cancer, cardiovascular disorders, viral diseases and metabolic disturbances. Once a miRNA binds to its target mRNA, protein translation is inhibited or the mRNA is degraded via the miRNA-mediated RNA interference. Over the earlier years, researchers have made great efforts to develop techniques appropriate for miRNA detection and quantification; a wide range of creative and innovative techniques (more than 30 different methods) have been developed and validated. The aim of this review is to discuss the basic concept of miRNA biogenesis and to present the most important miRNA expression detection methods with better understanding of strength and pitfalls of these techniques.

**miRNA and siRNA**

Before we introduce biogenesis of miRNA, it is important to know the difference between small interfering RNAs (siRNAs) and miRNA despite they share the same theme. These differences is presented in Table 1.

**Table 1. Comparison of miRNA and SiRNA** (adopted from Mack, 2007)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>siRNA</th>
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<tbody>
<tr>
<td>Occurrence</td>
<td>Occur naturally in plants and animals</td>
</tr>
<tr>
<td>Configuration</td>
<td>Single stranded</td>
</tr>
<tr>
<td>Length</td>
<td>21–22 nt</td>
</tr>
<tr>
<td>Complementary to target mRNA</td>
<td>Not exact, and therefore a single miRNA may target up to hundreds of mRNAs</td>
</tr>
<tr>
<td>Biogenesis</td>
<td>Expressed by genes whose purpose is to make miRNAs, but they regulate genes (mRNAs) other than the ones that expressed them</td>
</tr>
<tr>
<td>Action</td>
<td>Inhibit translation of mRNA</td>
</tr>
<tr>
<td>Function</td>
<td>Regulators (inhibitors) of genes (mRNAs)</td>
</tr>
<tr>
<td>Clinical Use</td>
<td>Possible therapeutic uses either as drug targets or as drug agents themselves. Expression levels of miRNAs can be used as potential diagnostic and biomarker tools</td>
</tr>
</tbody>
</table>
Biogenesis of miRNA

Transcriptional regulation has been proposed to be the major mechanism controlling tissue- and cell type-specific expression of miRNA. Genes for miRNAs are located in the chromosomes, and many of them are identified in clusters that can be transcribed as polycistronic primary transcripts. Some miRNAs are encoded by their own genes and others are encoded by the sequences as a part of the host protein-coding genes. On the basis of the genomic arrangement of their genes, miRNAs can be grouped into two classes.(6)

1. Intergenic miRNAs (miRNA-coding genes located between protein-coding genes).
2. Intragenic miRNAs (miRNA-coding genes located within their host protein coding genes). Further, the intragenic miRNAs can be divided into the following subclasses:
   A. Intronic miRNAs (miRNA-coding genes located within introns of their host protein-coding genes)
   B. Exonic miRNAs (miRNA-coding genes located within exons of host protein-coding genes)
   C. 3'UTR miRNAs (miRNA-coding genes located within 3'UTR of host protein-coding genes)
   D. 5'UTR miRNAs (miRNA-coding genes located within 5'UTR of host protein-coding genes)

A majority of miRNAs belong to intergenic and intronic miRNAs comprising ~42 and ~44% of the total, respectively, and the other three categories are rare, with the exonic miRNAs being ~7%, 3'UTR miRNAs being 1.5%, and 5' UTR miRNAs being 1%. Clearly, miRNAs either have their own genes or are associated with their host genes; miRNAs are generated by different mechanistic pathways. However, in general, biogenesis of miRNAs can be summarized as a five-step process(6,8-10) as illustrated in Figure 1.

- **Generation of primary miRNAs**: The intergenic or intronic miRNA genes are first transcribed as long transcripts, called primary miRNAs (pri-miRNAs), mostly by RNA polymerase II.
- **Generation of precursor miRNAs**: pri-miRNAs are processed to precursor miRNAs (pre-miRNAs) by the RNase endonuclease-III Drosha and its partner DGCR8 in the nucleus. These pre-miRNAs are ~60–100 nts with a stem-loop or hairpin secondary structure.
- **Nucleus to cytoplasm translocation of pre-miRNAs**: Pre-miRNAs then get exported to the cytoplasm from the nucleus through nuclear pores and nuclear export protein (exportin-5). After a pre-miRNA is exported to the cytoplasm, the pre-miRNA is released from Exp-5.
- **Generation of mature miRNAs**: In the cytoplasm, pre-miRNAs are further processed by Dicer, which is a highly conserved, cytoplasmic RNase III ribonuclease that chops pre-miRNAs into ~22-nt duplexes of mature miRNAs containing a guide strand and a passenger strand.
- **Formation of miRISC**: Mature miRNAs get integrated into a RNA-induced silencing complex (RISC) to form the miRNA-RISC complex (miRISC). Only one strand of miRNA/miRNA*, the guide strand, is successfully incorporated into RISC, while the other strand, the passenger strand, is eliminated. Strand selection may be determined by the relative thermodynamic stability of two ends of miRNA duplexes. The strand with less stability at the 5' end is favorably loaded onto RISC, whereas the passenger strand is released or destroyed. Argonaute proteins (Ago) are the key catalytic enzymes within the complex. Ago then interacts with miRNA and causes transialational repression or cleavage.

**Figure 1. Biogenesis of miRNA and siRNA (adopted from Meister, 2013)**

Canonical and non-canonical miRNA biogenesis pathways

Recently, it was found that pri-miRNAs are processed into mature miRNAs through canonical and non-canonical biogenesis pathways as illustrated in Figure 2. During canonical miRNA biogenesis, the pri-miRNA hairpin is processed into pre-miRNA by Drosha, a member of the RNase III family. While in non-canonical miRNA biogenesis, the pre-miRNAs are generated by miRNA splicing machinery, independent of Drosha-mediated digestion in the nucleus. In both pathways, the pre-miRNAs are exported to the cytoplasm via exportin 5 and further processed by a second RNase III enzyme, Dicer. The mature double-stranded miRNAs are then loaded into a functional ribonucleoprotein complex called the RNA-induced silencing complex (RISC), which serves as the catalytic engine for miRNA-mediated post-transcriptional gene silencing. RISC consists of multiple protein factors, and Argonaute proteins are the key catalytic enzymes within the complex. Argonaute proteins bind miRNAs and are essential for their downstream gene-regulatory mechanisms to regulate mRNA degradation and protein expression(11-13).
miRNA Action

Early studies of the C. elegans miRNA Lin4 showed that miRNAs acted through translational repression. However, it is now thought that miRNAs may act through several additional mechanisms, including inhibition of translation initiation, inhibition of translation post-initiation and induction of mRNA destabilization and decay. In mammalian cells, miRNA destabilization is thought to be the dominant mode of action of miRNAs, possibly involving P-body proteins. P-bodies are also known as cytoplasmic processing bodies, which are enriched with enzymes and other proteins involved in mRNA degradation and sequestration from translational machinery. P-body components, such as GW182 (also known as TNRC6A), RNA-decapping enzyme 1 (DCP1), DCP2 and the ATP-dependent RNA helicase p54 (also known as RCK and DDX6), have been found to physically interact with Argonaute proteins and are essential for miRNA-mediated gene repression. It is also worth noting that each miRNA can regulate multiple target mRNAs simultaneously. For some miRNAs, the targets are components of a single pathway, which suggests that miRNAs could be used to manipulate the activity of an entire pathway rather than the components alone. One such example is miR17 family miRNAs, which target components of the transforming growth factor-β (TGFβ) signalling pathway, such as TGFβ receptors, SMADs and the downstream effector gene cyclin-dependent kinase inhibitor 1A (CDKN1A; which encodes p21), as well as several other genes.

DISCOVERY OF miRNA AS THERAPEUTICS

miRNAs and miRNA-targeting oligonucleotides have several advantages over traditional small-molecule drugs, most notably the simplicity with which oligonucleotides can be chemically modified to enhance their pharmacokinetic/pharmacodynamic (PK/PD) profiles and the ability of miRNAs to target multiple genes simultaneously. Currently, miRNA-targeting therapies are an area of intense interest to many pharmaceutical companies, where many of such compounds are in preclinical and clinical development for a variety of indications (Table 2).

METHODS FOR ANALYZING miRNAs EXPRESSION

Owing to the uniqueness of miRNAs distinct from protein-coding mRNAs, there are different approaches to detect and quantify miRNAs and mRNAs based on the following properties:

1. The extremely small size of miRNAs renders most conventional biological amplification tools ineffective because of the inability of even smaller primers/promoters (8-to-10-nt) to bind on such small miRNA templates. For example, the regular RT-PCR can only be used to quantify miRNA precursors rather than the mature miRNAs.

2. The close similarities among family members of miRNAs have presented challenges for developing miRNA-specific detection assays.
Table: 2 Selected anti-miR therapeutics currently in development (adapted from Li and Rana, 2014)[13]

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Oligonucleotide format</th>
<th>Indications</th>
<th>Companies</th>
<th>Developmental stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR 122</td>
<td>LNA-modified antisense inhibitor</td>
<td>HCV infection</td>
<td>Santaris Pharma</td>
<td>Phase II</td>
</tr>
<tr>
<td>miR 122</td>
<td>GalNAc-conjugated antisense inhibitor</td>
<td>HCV infection</td>
<td>Regulus Therapeutics</td>
<td>Phase I</td>
</tr>
<tr>
<td>miR 34</td>
<td>mRNA mimic replacement</td>
<td>Liver cancer or metastasized cancer involving liver</td>
<td>miRNA Therapeutics</td>
<td>Phase I</td>
</tr>
<tr>
<td>Let 7</td>
<td>miRNA mimic replacement</td>
<td>Cancer (details undisclosed)</td>
<td>miRNA Therapeutics</td>
<td>Preclinical</td>
</tr>
<tr>
<td>miR 21</td>
<td>2′-F and 2′-MOE bicyclic sugar modified antisense inhibitor</td>
<td>Cancer, fibrosis</td>
<td>Regulus Therapeutics</td>
<td>Preclinical</td>
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<tr>
<td>miR 208</td>
<td>Antisense inhibitor</td>
<td>Heart failure, cardiometabolic disease</td>
<td>miRagen/Servier</td>
<td>Preclinical</td>
</tr>
<tr>
<td>miR 195</td>
<td>Antisense inhibitor (miR 15 family)</td>
<td>Post-myocardial infarction remodeling</td>
<td>miRagen/Servier</td>
<td>Preclinical</td>
</tr>
<tr>
<td>miR 221</td>
<td>Antisense inhibitor</td>
<td>Hepatocellular carcinoma</td>
<td>Regulus Therapeutics</td>
<td>Preclinical</td>
</tr>
<tr>
<td>miR 103/105</td>
<td>Antisense inhibitor</td>
<td>Insulin resistance</td>
<td>Regulus Therapeutics</td>
<td>Preclinical</td>
</tr>
<tr>
<td>miR 10b</td>
<td>Antisense inhibitor</td>
<td>Glioblastoma</td>
<td>Regulus Therapeutics</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

3. Small RNAs are less efficiently precipitated in ethanol and for this reason during the isolation by standard Trizol protocol of the RNA, resuspension in ethanol should be avoided.

4. miRNAs seem to be more stable than longer RNAs, for example in degraded samples it is still possible to obtain readable miRNA expression data. Moreover, miRNAs have a higher stability compared to mRNAs in samples obtained from formalin-fixed paraffin-embedded tissues or in serum.

Ideal Methods for miRNA Detection

The ideal miRNA profiling method should fulfill several requirements:

- Sensitive enough to determine miRNA profiles even with small amounts of starting material
- Specific enough to reproducibly detect a 1-nt difference between miRNAs
- Able to provide quantitative analysis of miRNA levels
- Capable of processing multiple samples in parallel
- Easy to perform and not require equipment or reagents not readily available in a conventional molecular biology laboratory.[38]

Classification of Methods for miRNA Detection

To date, several methods have been developed for miRNA detection. Currently available methods can be classified into following categories:

Methods based on the mechanism of miRNA capturing

- Hybridization-based techniques (e.g., Northern blots, in situ hybridization, RT-PCR, and microarrays).
- Amplification-based techniques (e.g., real-time quantitative PCR; gold nanoparticle-initiated silver enhancement).
- Cloning-based techniques (e.g., miRAGE).

Methods based on throughput of miRNA profiling

- Low throughput methods (e.g., Northern blots, in situ hybridization).
- Medium throughput methods (e.g., multiplex RT-PCR, miRAGE).
- High throughput methods (e.g., microarrays, deep sequencing).

Methods based on quantification

- Quantitative (e.g., real-time quantitative PCR).
- Semi-quantitative (e.g., Northern blots).
- Non-quantitative (e.g., Northern blots, in situ hybridization).

Methods based on miRNA capture probes

- One-probe assays (e.g., Northern blots, in situ hybridization, and microarrays).
- Two-probe assays or sandwich-type assays (e.g., gold nanoparticle-based assays and enzyme-amplified assays).

Methods based on read-out format

- Optical signal detection (including a variety of biochemical and chemical ligation-based techniques and PCR-based assays that use colorimetry, fluorescence, and bioluminescence).
- Electrical signal (e.g., polyaniline nanowire technique; electrocatalytic nanoparticle tags technique).

Methods based on miRNA labeling

- Fluorescence labeling (e.g., Taqman real-time RT-PCR).
- Luminescence labeling (e.g., electrocatalytic moiety labeling technique).
- Non-labeling (e.g., electrocatalytic moiety labeling technique).

These above methods have all achieved a certain level of success and have been successfully applied to generate miRNA transcriptomes for our understanding of the biological importance of miRNAs in various pathophysiological settings, even though none of these methods is perfect and has inherent limitations. Some methods rely on expensive equipment and an advanced read-out system, which might limit their application.

Which tool is better?

The best choice depends on the application. It is a balance of cost, precision, accuracy and sample quantity. If the purpose...
is to screen a bunch of samples to find a few microRNAs that change and you can tolerate a false negative, then the microarray may be the best platform. If the purpose is to detect microRNAs where the sample amount is limiting, then qPCR has better sensitivity, and if you are trying to see different isoforms or very similar microRNAs, then sequencing is going to be the best approach.\(^{(39)}\)

Herein, we introduce the most popular and currently in use methods for miRNA detection. The principle of qRT-PCR, miRNA microarray and nanosorting, and RNA sequencing is depicted in Figure 3. In TagMan qRT-PCR (3a), the reverse transcription reactions use stem–loop primers that are specific to the 3’ end of the microRNA (miRNA) for specificity (top left, 3a). Amplicons are generated using an miRNA-specific forward primer and a reverse primer. As the DNA polymerase proceeds along the template, the TaqMan probe is hydrolysed and fluorescent dye is freed from the quencher, resulting in light emission (top middle). In SYBR-green-based qRT-PCR, miRNA is typically polyadenylated at the 3’ end, and oligo-d(T) is used as a reverse transcription primer (bottom left). An miRNA-specific forward primer and a reverse primer that anneals to the 3’ portion of the miRNA sequence as well as to the poly(A) tail enable PCR amplification with dsDNA-intercalating SYBR green dye as the detector (bottom middle). Both TaqMan and SYBR-green-based qRT-PCR are available in ‘array’ format (right). In miRNA microarray (3b).

DNA-based capture probes (which may or may not incorporate LNA-modified bases) are used to capture fluorescently tagged miRNAs; this is followed by scanning of slides and quantification of fluorescence. Nanostring nCounter (3c). A bridge oligonucleotide templates ligation of a miRNA to a specific tag. Capture and detection is done by two target-specific probes: a 3’ capture probe containing biotin to allow adsorbance to the solid phase via streptavidin and a second 5’ reporter probe with an individual colour-coded sequence. No amplification or labelling of miRNA is required with this method. The last one is the RNA-seq (3d). Currently established miRNA-
seq methods begin with reverse transcription of miRNA into a cDNA library. Adaptor ligation then allows the library either to be affixed to a solid phase, as in the Illumina platform, or to beads for emulsion PCR, as in the Roche and ABI platforms for details of sequencing chemistry. A ‘decision tree’ is provided to assist in choosing an appropriate profiling platform is also illustrated in Figure 3. Today’s several commercial vendors’ offerings of microRNA profiling technology is listed in (Table 3). Though specific products and techniques vary, researchers should be aware of the relative strengths and weaknesses of the platforms.

**Figure 4. miRNA-profiling hierarchy.** *The cost of RNA sequencing (RNA-seq) is rapidly dropping with newer platforms and sample multiplexing using DNA barcoding, qRT-PCR, quantitative reverse transcription PCR (adopted from Pritchard et al., 2012).({}^{40})*

<table>
<thead>
<tr>
<th>Company</th>
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<td>454 Life Sciences</td>
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<td>Advanced Array Technology (now Eppendorf Array Technologies)</td>
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**Table 3. Suppliers guide: Companies offering microRNA profiling technology** (Baker, 2010)({}^{39})

**FUTURE PERSPECTIVES**

Better understanding of the role miRNA in gene regulation and disease will help to unravel the principles and causes of diseases. Furthermore, studying of miRNA in different compartments or miRNA present in different complexes will be useful for miRNA profiling of specific subcellular compartments rather than extracts from whole cells. It is expected that in coming years, the acquired biological information about miRNAs can be broadly translated into the clinical use. Currently, microRNAs offer an attractive choice as stable biomarkers for cancer detection, diagnosis, and prognosis assessment in both the tumor tissue and circulating blood thus miRNAs hold great potential as diagnostic markers and therapeutic targets despite of major challenges that still need to be overcome (e.g., tissue-specific delivery). Additionally, improvement of the current detection techniques on sensitivity, specificity and easy applicability is still ongoing.

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Dr. AL Zaharna Mazen is an assistant professor at Islamic University of GAZA. He received his Bachelor and Master degree in Medical Technology form University of Malta and The Islamic University of Gaza-Palestine, respectively. Mr. Al Zaharna graduated from CityU of Hong Kong. His main interest on the effect of bioactive phytocompounds on cancer cells at molecular level. Dr. CHEUNG Hon-Yeung, who is an associate professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong since 1989, is a manufacturing pharmacist and biotechnologist. He has more than 40 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 220 papers and articles in many prestigious international journals. His email address: bhhonyun@cityu.edu.cn.hk

References
24. Lytle JR, Yario TA and Steitz JA. (2007). Target miRNAs are repressed as efficiently by miRNA-binding sites in the 5' UTR as in the 3' UTR. Proceedings of the National Academy of Sciences of the United States of America, 104:9667-9672.
Keeping: 0% HBV resistance profile for 8 years\textsuperscript{1,2}

Leading: potent HBV suppression for 8 years\textsuperscript{1,2}

Running: well tolerated throughout the 8-year follow up period\textsuperscript{1,2}
Blood-tonifying and Regulating Functions of Paeoniae Radix (Shaoyao)

ZHAO, Hangb, b; LI, Lingqui; CHEUNG, Hon-Yeungb*

a School of Public Health, Guangdong Medical University, Dongguan, China
b Department of Biomedical Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Hong Kong SAR, China
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ABSTRACT

Paeoniae Radix is a medicinal herb derived from the dry root of Paeonia lactiflora Pall or P. veitchii Lynch. The herb is named either as Paeoniae Radix Alba (PRA) or Paeoniae Radix Rubra (PRR) depending on its color, origin and method of processing. Both PRA and PRR are commonly used traditional Chinese medicines (TCMs), whose medicinal uses could be dated back to the Northern and Southern Dynasties (AD 420-589). Although the content of individual component is different, the overall chemical profile of these two TCMs is similar. This review summarizes their sources, chemical compositions, biological functions and clinical applications of PRR and PRA; aiming to explore their medicinal uses based on scientific data derived from chemical analysis and pharmacological studies.

Keywords: Peony root, Paeoniae Radix Alba, Paeoniae Radix Rubra, Paeonia lactiflora Pall, Paeonia veitchii Lynch, Paeoniflorin, Blood-tonifying medicinal, Blood-regulating medicinal

DESCRIPTION AND BACKGROUND

Historical background of Medicinal Uses of Paeoniae Radix

Paeonia Radix, also known as Shaoyao, is one of the commonly used Traditional Chinese medicines (TCMs) with many claimed functions. It is the peeled and dried root of either Paeonia lactiflora Pall (PLP) or P. lactiflora var. trichocarpa, which frequently referred as Paeoniae Radix Alba (PRA, baishao in Chinese), or from P. veitchii Lynch, which is referred as Paeoniae Radix Rubra (PRR, chishao in Chinese) (Figure 1). These perennial flowering plants are belong to the Ranunculaceae (Buttercup) family. There are about 35 species of plants in the Paeonia genus and all of them distribute in the temperate regions of Euasia. In China, only about 11 species can be found.

Although both PRA and PRR are resemble to each other in term of botanical origin, i.e. similar genetic background and secondary metabolism pathway, these two herbal substances are recommended for different clinical uses according to Chinese Pharmacopoeia (Table 1). Baishao, in general, is regarded as a blood tonifying and replenishing medicine while chishao is a blood-activating and stasis-dispelling medicinal. The classification of Paeoniae Radix, however, is not without any arguable views.

During the Northern and Southern Dynasties (420-589), PLP had already been classified as red or white and in the Tang and Song Dynasties, although PLP was also grouped into red or white based on the colors of the root, the classification was obscure. From the Yuan to Qing Dynasty, PLP was identified according to the color of the flower. Overall speaking, PRA is mainly cultivated in Hanzhou (Anhui province), Hangzhou (Zhejiang province), and Heze (Shandong province) while most PRR is wild and it is mainly found in Inner Mongolia, Heilongjiang, Jilin and Liaoning. The herb is the dried root of peony root based on Chinese Medicines

<table>
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<tr>
<th>Indication for Baishao (PRA)</th>
<th>Indication of Chishao (PRR)</th>
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<td>Blood deficiency and sallow complexin</td>
<td>Heat enterling nutrient-blood aspects</td>
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<td>Menstrual irregularities</td>
<td>Macula and papula caused by warm toxin</td>
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<td>Spontaneous sweating</td>
<td>Hematemesis and epistaxis</td>
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<td>Night sweating</td>
<td>Red painful swelling eyes</td>
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<td>Hypochondriac pain</td>
<td>Liver depression with hypochondriac pain</td>
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<td>Abdominal pain</td>
<td>Amenorrhea and dysmenorrhea</td>
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<td>Spasm and pain of limbs</td>
<td>Abdominal pain caused by aggregation and accumulation</td>
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<td>Headache and dizziness</td>
<td>Injuries from falls</td>
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<td>Sore and ulcer</td>
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The medicinal uses of PLP can be dated back 2000 years ago. In the Treatise on Fevers and Other Diseases (傷寒論)
written by Zhongjing Zhang in the Dong Han Dynasties, 31 prescriptions based on the use of PLP had been recorded. These prescriptions indicated the treatment of yin, yang, biao, li, han, re, xu, and shi (Chinese terms for describing the health conditions of human) etc. More descriptions of its medicinal application of both PRR and PRA were subsequently documented in Tai Ping Sheng Hui Fang and Sheng Ji Zong Lu, both of these were written by a Chinese herbalist in ancient time.(8,9) In Ben Cao Qiu Zhen, the author claimed that although their major functions are similar, the white one (PRA) can restrict yin and mediate yingqi (a term of Chinese medicine — vital essence from various nutrient), while the red one (PRR) can only clear toxicants from blood. However, the major functions of PRA and PRR are similar according to Ben Cao Bei Yao and Zhu Jie Bei Yao. It was reported that PRR is more effective in purging liver fire, while PRA nourishes vitality.(10)

**Figure 1. A photograph of Peony and its medicinal materials.** Plat 1: Flowering plants of Paeonia lactiflora Pall (P. albilflora Pall); 2: Decortion pieces of Paeoniae Radix Alba (baishao); 3: Flowering plants of Paeonia veitchii Lynch; 4: Decortion pieces of Paeoniae Radix Rubra (chishao)

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### CHEMICAL COMPOSITION OF PRA AND PRR

In recent years, the chemical composition of both PRA and PRR has been intensely studied. Components isolated from these two TCMs are mainly monoterpenoid glucosides and polyphenols. In the former, it contains mainly paeoniflorin, albiflorin, benzoylpaeoniflorin and Oxypaeoniflorin etc. For the latter, it contains gallylglucose and paeonol.(11, 12) According to data in some literatures, the contents of paeoniflorin (Figure 2), albiflorin (Figure 2) and catechinic acid in these two herbs varied significantly.(13, 14) Fan’s(15) group utilized 1H-NMR metabonomics technique analyze PRA and PRR. They reported that abundant quantity of arginine, threonine, aspartic acid, glutamine, gabaron tea, citric acid, succinic acid, lactic acid, albiflorin std, galloylpaeoniflorin, 1,2,3,3,4,6-Pentagalloylgucose and gallic acid was found in PRA while a higher content of alanine, α-glucose, sucrose, paeoniflorin, catechinic acid, β-sitosterol, fatty acid, and paeonol was found in PRR. Overall speaking, it can be concluded that the major components of both types of Peony are identical with different proportion. The variation in different component may be attributed to genetically differences, and/or the way of cultivations.(16,17)

**Distribution of Bioactive Components in different part of Peony**

Li and his associates analyzed paeoniflorin and benzoic acid in different parts of PRA and PRR by means of HPLC.(18) They reported that the content of paeoniflorin in the following descendant order: main root of PRR, main root of PRA, leaf, stem and flower. They had also found that different growing environment and processing methods could result in different chemical component and composition ratio, even although the same plant and the same part were analyzed. Besides, ethanol extraction of wild herb gave a better yield in chemical contents while cultivated herb was found to have less contents and varieties of components. This study suggests that wild herb might contain more bioactive substances in comparison to the cultivated one.(19)

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**Figure 2. Chemical structure of (i) Paeoniflorin and (ii) Albiflorin**
Effect of Processing on Chemical Composition of Paeonia Radix

Effect of Sulphur Fumigation on Chemical Composition

Sulphur-fumigation is one of several methods commonly used for preventing against insects and moulds during post-harvest handling of medicinal herbs. Some recent investigations have proven that sulphur-fumigation could cause chemical transformation of Paeoniae Radix Alba. Ming Kong found that sulphur-fumigation not only changed the proportions of bioactive components, but also caused reduction of quality consistency of both raw materials and aqueous decoctions of PRA. Song reported that the main effective constituents in Guizhi Decoction were changed if sulphur-fumigated PRA was used.

A HPLC fingerprint method has been developed by Wang to evaluate the qualitative differences on sulphur-fumigated and non-fumigated PRA. His study revealed that fumigated PRA turned white and had its unique fragrance disappeared along with the production of pungent sour gas. Hence, he concluded that fumigation had significant effect on the paeoniflorin content and this process could influence the efficacy of Paeoniae Radix Alba.

Effect of Processing Method on Chemical Composition

As processing method has been reported to change the content of chemical composition in Paeoniae Radix Alba, the content of some effective ingredients in Paeoniae Radix Alba could be affected by drying. A preferable method, that crude drug should be cooked before peeled and dried, was recommended. As a matter of standardization of processing, it is suggested that PRA should be peeled and sliced before being dried. In this method, peony roots are put into boiled water for ten minutes and sliced into 2-3 mm in thickness after peeling the root bark. The slices are then dried at 55°C in an oven, which was similar to the traditional processing method adopted by suppliers in Bozhou.

Peeling, boiling and drying might affect the contents of albinorin, paeoniflorin, benzoyl paeoniflorin and paeonol on several aspects. No matter before and after processing, the contents of both paeoniflorin and peony lactone glycosides have been found significantly different in PRA. A qualitative analytical method of liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (HPLC-Q-TOF-MS/MS) has been developed for identification of multi-constituents and an HPLC-DAD analytical method has been developed for simultaneously determining 14 major compounds (gallic acid, protocatechuic acid, paeoniflorin sulfonate, protocatechuic aldehyde, methyl gallate, oxypaeoniflorin, catechins, albinorin, and paeoniflorin, ethyl gallate, benzoic acid, pentagalloylglucone, benzoyl-paeoniflorin, and paeonol) in PRA and PRR. With this quantitative method, it was found that contents of 8 ingredients were different between PRA and PRR.

Quality Control of PLP

To obtain PLP of reliable quality, Bozhou in Anhui province is traditionally regarded as the authentic site for cultivation of PRA. However, growing environment and climate have been reported to influence their quality. Zhang and his group established the UPLC-MS/MS fingerprint of PRA and found 7 peaks, in which 4 peaks was confirmed with the help of reference substances or molecule. When they studied the similarity of 11 batches from different area, the similarity index was 0.9 indicating a good consistency. Besides, an assay method has also been developed for effective determination of the content of paeoniflorin, albinorin, benzoyl paeoniflorin and paeonol in Paeoniae Radix Alba. Hence, instrumental methods for the quality control of Paeoniae Radix are available.

MODE OF MEDICINAL ACTIONS OF PÆONEIAE RADIX

Modern pharmacological studies reveal that both Paeoniae Radix Alba and Radix Paeoniae Rubra have many common pharmacological effects such as anti-inflammatory, anticoagulant, immune regulation. When metabolomics approach was applied to study the influence of PRA and PRR on the metabolic changes in rats with acute liver injury, it showed that protective effect of these two TCMs against liver injury was about the same. Although in the practice of Chinese medicine, PRR is regarded as a kind of heat-clearing drug, which can clear heat, cool blood, and remove blood stasis while PRA can tonify deficiency, restrict yin, stop sweating, nourish liver, relieve pain, and enrich blood. There was no clinical data to support the claim. Only a specific and sensitive HPLC-ESI-MS methods has been developed for simultaneous determination of paeoniflorin, albinorin and oxypaeoniflorin in rat plasma. Chao Feng reported that the absorption of paeoniflorin, albinorin and oxypaeoniflorin were significantly different and therefore, the pharmacokinetic characteristics of these two herbal substances was different. His findings were useful for further study on the pharmacological effects of Paeoniae Radix Alba and Paeoniae Radix Rubra.

Pharmacological effects of PRA

According to Chinese medicines, Paeoniae Radix Alba is used to calm liver-wind, relieve pain, nourish blood, regulate menstrual functions, suppress sweating, anti-inflammatory, improve the body's anti-inflammatory process, and also be used in rheumatoid arthritis. This herb relaxes vascular smooth muscle via endothelium-dependent and Akt- and SOCE-eNOS-cGMP-mediated pathways through activation of both KCa and KATP channels and inhibition of L-type Ca2+ channels. PRA can dose-dependently inhibit time-dependent cell responses, and have anti-allergic function in vitro and in vivo.

PRA not only suppressed mast cell degranulation via inhibiting the translocation of granules to the plasma membrane, but also blocking membrane fusion and exocytosis. It may be other anti-allergic components present in addition to paeoniflorin because PRA regulated mast cell activation with multiple targets. When large dose of paeoniae radix alba was applied, it generated a good effect to treat renal colic, indicating that PRA has spasmylosis analgesic effects. Furthermore, albinorin is a potential anti-depressant glycosides. It produced significant antidepressant-like effects, which are closely related to the action of hippocampal; with which 5-HT/NE increased and BDNF expressed. The compatibility of two Chinese herbs could alter the pharmacokinetics and tissue distribution properties of major bio-active components in the single herb.
Compared with single herb, RRHC could increase or decrease the concentrations of five components at different time points compared with the single herb.\(^{[52]}\)

**Pharmacological effects of PRR**

Modern pharmacological studies have shown that Paeonieae Radix Rubra has many pharmacological effects. In Xiang’s experiment, increased blood capillary leakage in mouse abdominal cavity and body weighing test were carried out. It turned out that PRR is anti-inflammatory and can remove pain.\(^{[53]}\) Chishao has a function of blood invigoration which has been used in severe cholestatic hepatitis for decades and obtained satisfactory effects. As a promising novel treatment approach, widely using large dosage of Chishao in formulae may enhance the curative efficacy for cholestatic hepatitis.\(^{[54]}\) Huoxue-Tongluo lyophilized powder for injection (HTLPI), a traditional Chinese medicine preparation, is a compound of Persicae semen and Paeonieae Radix Rubra that is used mainly for treating blood-stasis obstruction syndrome in the acute stage of cerebral ischemic stroke.\(^{[55]}\) Paeoniae radix rubra (APE) has a function for anti-atherosclerotic effects. Paeonieae radix rubra (APE) extract had protective effect against liver fibrosis in mice. In the further study, the mechanism of action of APE is hypothesized to proceed via scavenging free radicals, decreasing TGF-β1 levels and blocking of the TGF-β/Smad signaling pathway.\(^{[56]}\) In addition, Total glycosides of Radix paeoniae rubra have some anti-tumor effect in vivo, which might have been accomplished through the regulation of the immune system.\(^{[57]}\)

**Pharmacological effects of Paeoniflorin**

Total glycosides of paeony is a mixture of compounds which were water or ethanol extract of Paeoniflorin Radix Alba, of which paeoniflorin is the major active component.\(^{[58],[59]}\) Paeoniflorin is a common composition in Paeonieae Radix Alba and Paeonieae Radix Rubra. The Chinese Pharmacopoeia specified that glycosides in Paeonieae Radix Rubra should not be less than 1.8% while glycosides in Paeonieae Radix Alba not less than 1.6%.\(^{[60]}\) Paeoniflorin has many medicinal uses; some literature suggested that it can reduce the damage to the skin due to type B ultraviolet. Exposure to UV induces extensive generation of ROS, and results in photo-aging and development of skin cancer. In recent years, naturally occurring herbal compounds have gained considerable attention as protective agents for UV exposure. Paeoniflorin (PF), which is isolated from peony root (Radix Paeoniae Alba), is a novel natural antioxidant. The protective effects of PF on UV-induced skin damage in vitro had been investigated. It has been demonstrated that the protective effects of PF were mediated via the ROS-p38-p53 pathway. In addition, cell death analysis has demonstrated that PF treatment markedly reduced UV-B-radiation-induced apoptosis in keratinocytes, which was accompanied by increased procaspase 3 expression and decreased cleaved caspase 3 expression. Treatment with PF markedly reduced the production of ROS, and inhibited the activation of p38 and p53 in human keratinocytes, suggesting that the ROS-p38-p53 pathway has a role in UV-B-induced skin damage. In conclusion, PF was able to attenuate UV-B-induced cell damage in human keratinocytes.\(^{[60]}\)

PF had been used to treat psoriasis. It alleviated IMQ-induced keratinocyte proliferation and inflammatory cell infiltration, and reduced mRNA levels of Th17 cytokines at day 4 and phosphorylation of Th17 differentiation-related proteins.

Two and 20 μg/mL PF were found inhibitory to mRNA expression of Th17 cytokines and phosphorylation of Stat3 in spleen cells under Th17 polarizing conditions. This means that PF inhibits IMQ-induced psoriasis by regulating Th17 cell response and cytokine secretion via phosphorylation of Stat3.\(^{[61]}\)

PF inhibited TNF-α-induced expression of ICAM-1. Compared with the normal group, ICAM-1 protein expression levels obviously increased (P < 0.01). Compared with the TNFa group, ICAM-1 protein expression levels were obviously inhibited in the high dose PAE group (P < 0.05). Protein expression levels of ph-p38 and ph-ERK were obviously higher in the high dose PAE group (P < 0.05). Compared with the normal group, IkBo protein expression levels obviously decreased in the TNF-α group (P < 0.01). Compared with the TNFa group, TNF-α-induced IkBo degradation could be significantly inhibited in the high dose PAE group (P < 0.01); the inhibition of PAE on IkBo degradation could be significantly inhibited in the SB group (P < 0.05). NF-κB/p65 signal was mainly located in cytoplasm in the normal group. NF-κB/p65 was translocated from cytoplasm to nucleus after stimulated by TNF-α for 45 min in the TNFa group, while it could be significantly inhibited in the high dose PAE group. Its action might be associated with inhibiting TNFR1/NF-κB signaling pathway, p38 participated and mediated these actions.\(^{[62]}\)

Some literature indicated that paeoniflorin plays an important role of anti-depressant, its mechanism may be involved in monoaminergic nervous system.\(^{[63]}\)

**Pharmacological effects of albiflorin**

The effect of enriching blood of paeoniflorin and albiflorin on blood deficient mice model induced by radiation indicating that paeoniflorin and albiflorin are active constituent in blood-enriching effect.\(^{[64]}\) To explain paeoniflorin’s blood-enriching function, it is postulated that paeoniflorin promotes the secretion of hematopoietic factor and mediates the hyperplasia and differentiation of hematopoietic cell.

Besides, paeoniflorin can regulate the negative hematopoietic factor, and reduce its suppression on hematopoietic function.\(^{[65]}\) In the restraint combined with cold and hot stimulation model, albiflorin can up regulate the expression of monoamine neurotransmitter, and therefore has anti-depression function.\(^{[66],[67]}\)

**UNDESIRABLE EFFECTS**

Although a statement made by Ward (2005) in “The Essential Guide to Clinical Safety” claimed that baishao and chishao were hepatotoxic based on one reference that had been incorrectly interpreted, there is still a lack of incidence data to fully substantiate this conclusion. Nevertheless, blistering of mouth and throat, diarrhea, dizziness, fainting, gastroenteritis, hematuria, nausea, salivation, stomach pain and/or vomiting may occur according to Ahmad et al (2012).
INTERACTION WITH CONVENTIONAL DRUGS

Antiepileptic medicinal products may have a high chance to interact with concomitant medications of Peony extract. The administration of phenytoin in combination with Paeoniae Radix increased phenytoin Tmax 3-fold in rat model and this attributed to a delay of phenytoin absorption.

In the Alternative medicine Review (2001), it was assumed, peony may not be combined with antibiotics. Damage to the gut flora by antibiotics might interfere with the process for cleaving the aglycons of peony and theoretically decreasing peony’s efficacy.

MODE OF ADMINISTRATION

Peony Root (PR) is available as crude materia medica or extractives. It is commonly mixed with other herbal substances to become a compound Chinese medicine.

Dosage

3 – 15 g

CONCLUSION

PRR and PRA are similar in origin and chemical composition but their functions and clinical applications are somehow different due to different processing method and growing environment. Up to now, there are a few papers reporting the comparison of these two TCMs. However, no one can precisely differentiate them based on their functional differences, although some believe that the differences are attributed to the content disparity of paeoniflorin and albiflorin. Some more researches are certainly necessary before a clear picture can be seen.

ACKNOWLEDGEMENTS

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References


INTRODUCTION

The opportunity of attending the Forbidden City Pharmacist Forum in Beijing in May 2016 as well as the hospital visits was a fruitful experience for us as pharmacy students. It has provided us more holistic perspectives on different aspects of pharmacy; we are enlightened on the level of advancement and the extent of development potential for this professional pharmacy field. During the hospital visit, we have acknowledged the difference in pharmacy practice between mainland China and Hong Kong and that the learning from these differences could definitely help us to see better improvement areas in the health system for both China and Hong Kong.

Day 1: Hospital Visit

Before heading to the Forbidden City International Pharmacists Forum 2016, we had a chance to visit the China-Japan Friendship Hospital in Beijing. During our three hours stay in China-Japan Friendship hospital, we were made aware of the differences in pharmacy practice happening in Hong Kong and Beijing.

We visited the China-Japan Friendship Hospital on the first day of our trip. We were brought to visit the inpatient pharmacy. Since China-Japan Hospital is a large acute general hospital with 1900 beds, large volume of prescriptions were sent to the pharmacy every minute and hence everyone was very busy there. In addition, we noticed there was a huge cupboard with many baskets in it. Each basket has a tag with the name and location of the ward. That indicates where the drugs in that particular basket should be delivered. It seems to us that such a system could definitely increase the efficiency of drug delivery in the hospital.

Our next stop was the satellite pharmacy. Clinical Pharmacists at the satellite pharmacy would walk with doctors during the ward rounds and explain side effects of drugs to nurses and patients to resolve their worries. As Doctors may only be concerned about the therapeutic outcome without thoughtful consideration on the drug dosage or administration method. Hence, the clinical pharmacists could play a moderator role between nurses and doctors.

Apart from the inpatient pharmacies, we also had the chance to visit the Chinese medicine pharmacy. It was an eye-opening experience since Chinese medicine pharmacy is rarely seen in Hong Kong. We were told that after the herbs were picked according to the prescriptions, they were put into a big white bag and taken to brew in a pot with water until they were ready to be drunk by the patients. Since making the herbal medicine took time, instead of making one dose, doses for 3 days were made each time. It was a great chance for us to gain a deeper insight on how Chinese Medicine pharmacy worked in China.

Lastly, we visited the outpatient pharmacy, where the pharmacists introduced their new technology to us. There were automatic dispensing machines that help pick and dispense the drugs, which increased the efficiency and reduced the dispensing errors in outpatient pharmacy. The design of the outpatient pharmacy is very different to Hong Kong public hospital pharmacy. There were less pharmacists and dispensers as they depended more on the machines to handle the workload as compared to Hong Kong.

Day 2-3: Pharmacy Conference

The International Pharmacist Forum held in Jiuhua Shanzhuang, located in north part of Beijing was an eye-opening experience to every one of us. Through attending...
multiple seminars, each of us was given a bigger picture of the pharmacy practice industry as we tried to understand it from different perspectives. Below, we would share some interesting topics which we had come across.

The first seminar which caught our eye was about the practice of pharmacy service at the A&E Department in Taiwan. Due to limited manpower and resources, not all necessary medicine are available at the A&E pharmacy. Hence, there is a need to review the A&E pharmacy store once a year to replace less commonly used medicine with more commonly used ones to improve efficiency.

This was followed by a presentation on the cost effectiveness of aspirin in preventing ischemic stroke, myocardial infarction and colorectal cancer. The research was carried out by a master student from Singapore National University based on the calculation on Incremental Cost-Effectiveness Ratio. She adopted both one-way sensitivity analysis and probability sensitivity analysis and reached a conclusion that aspirin prevention is more cost-effective among males than females.

We also grasped the opportunity to understand more about Chinese herbal medicine. One of the speakers clarified on the misconception related to herbs induced liver injury (HILI). Up till present, no solid conclusion could be made on whether Chinese herbal medicine would lead to liver injury as arguments in support of this view did not seem to be sound. Some Chinese herbs were discovered to be mixed with Western medicine and it was difficult to determine which true causative agent for liver injury was. Further studies show that some HILI cases were temporary and the condition could be recovered once the medication was ceased.

As the main theme of this conference is internet and pharmaceutical care, there was a wide range of discussion on the usage of advance technology as an aid to improve pharmaceutical care. Patients failing to follow medication schedule has been a major obstacles in medication treatment. This problem is often induced by several factors including patients’ forgetfulness to follow medication schedule or their concerns not being addressed while their medications were dispensed.

We attended a section of the conference which had specifically addressed the above problems with the aid of information technology. For example, a pharmacist has created a mobile application which reminds patients to take medication at the correct time as well as incorporating a chat box between the patient and the pharmacist, so that the patient can ask questions regarding the drug treatment at any moment. Though this might not be the most innovative design, the consideration to deal with the patient’s need had amazed us. It had really allow us, as future pharmacists, to understand the importance to think from the points of view of a patient.

In addition, the use of information technology had also taught us the importance to learn how to rely and trust advanced technology. Though technology might not be able to take over all the work load of a pharmacist, for example checking any fault in the drug treatment or perform counselling with patient. Yet, it definitely was our good partner. Learning to share some of the workload using IT could really allow pharmacist to be more task focus, hence leading to a higher efficiency and could also reduce the potential chances of errors and faults.

Furthermore, we were also enlightened to the fact that information technology could be our good helping hands in improving pharmaceutical care, especially in providing sufficient information to the patient as well as patient data handling. Having seen how other areas apart from pharmacy knowledge was also applicable and might even be crucial to pharmaceutical service; we were enlightened that we should always widen our scope of knowledge and try to link different areas of knowledge to pharmaceutical care. Knowledge that might be seemingly unrelated could also be good helping hands for a pharmacist. As a whole, we had understood how important it was for us, as students, to increase our exposure to different aspects.

Personal Reflections

After attending the Forbidden City International Pharmacist Forum which had provided massive insights for our future career path, we also acknowledged and recognized the differences in pharmaceutical care between Hong Kong and mainland China. There was no doubt that mainland China had rapid developments over the past decade with improved technology and employment of elite professionals into the healthcare system. The main theme of the forum this year was internet and its relationship with pharmaceutical care. In our opinion throughout the hospital visit mainland China was completing their part with satisfactory outcomes. Perhaps due to considerable availability of resources and manpower, mainland China was able to maximize their hospital amenities to facilitate the servicing of their fast-growing population. At a macroscopic level, the development of pharmacist perspective towards a patient care oriented path is more than accepted by the general public, and with the help of technology, dispensing and simple manual processes in the past could be reduced. With only little knowledge at the start of the trip, we gained knowledge not only about the development of technology in mainland China, but also with improvements that the Hong Kong healthcare system could work on, for example introducing medication educational applications on mobile phones or even introducing them to hospitals. Concepts such as presenting mobile platforms for checking and recording drug dispensing were also new to public hospitals in Hong Kong, but would also be efficient if such platforms were further worked on.
Non-invasive Prenatal Testing: From Dream to Reality
Pharmacist Clinic in Pain and Palliative Care – Clinical Sharing
Innovative Measures of Cost-effectiveness of Pharmacy Services
Recognition of Mild Cognitive Impairment and Preventive Measures to Delay Progression
Application of the BEERS, START/STOPP Criteria in Practice Setting
Precision Medicine – Determining Treatment Options in the New Era

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PHARMACY
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2017 18-19 FEBRUARY
HK Convention & Exhibition Centre
Contraindication:
Hypersensitivity, including severe allergic reactions to yeast (a vaccine component), or after a previous dose of GARDASIL 9 or GARDASIL.

Precautions:
GARDASIL 9 is not intended to be used for treatment of active external genital lesions; cervical, vulvar, vaginal, or anal cancers and will not protect against diseases that are not caused by HPV.

Appropriate medical treatment should always be readily available in case of rare anaphylactic reactions following the administration of the vaccine. Observe symptoms of syncope (fainting), especially in adolescents and young adults, for approximately 15 minutes after administration of GARDASIL 9.

The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. Low-grade fever itself and mild upper respiratory infection are not generally contraindications to GARDASIL 9.

Individuals with impaired immune responsiveness, whether due to the use of immunosuppressive therapy, a genetic defect, Human Immunodeficiency Virus (HIV) infection, or other causes, may have reduced antibody response to active immunization.

GARDASIL 9 should be given with caution to individuals with thrombocytopenia or any coagulation disorder because bleeding may occur following an intramuscular administration in these individuals.

Drug Interactions:
GARDASIL 9 may be administered concomitantly with a combined booster vaccine containing diphtheria (d) and tetanus (T) with either pertussis [acellular, component] (ap) and/or poliomyelitis [inactivated] (IPV) (dTap, dT-IPV, dTap-IPV vaccines) with no significant interference with antibody response to any of the components of either vaccine.

Use of hormonal contraceptives did not appear to affect the type specific immune responses to GARDASIL 9. Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune responses to GARDASIL 9.

Adverse Reactions:
The most common (≥10%) local and systemic adverse reactions reported:
Headache, dizziness, nausea, pyrexia, and fatigue. Injection-site pain, swelling, erythema, pruritus, and bruising.

Pregnancy and Lactation:
Vaccination should be postponed until completion of pregnancy. GARDASIL 9 can be used during breast-feeding.

Forensic Classification:
P1S1S3

Active ingredient:
Each 0.5-mL dose contains approximately 30 mcg of HPV 6 L1 protein, 40 mcg of HPV 11 L1 protein, 60 mcg of HPV 16 L1 protein, 40 mcg of HPV 18 L1 protein, 20 mcg of HPV 31 L1 protein, 20 mcg of HPV 33 L1 protein, 20 mcg of HPV 45 L1 protein, 20 mcg of HPV 52 L1 protein, and 20 mcg of HPV 58 L1 protein.

Presentation:
GARDASIL 9 is a suspension for intramuscular administration available in 0.5-mL single-dose vials and prefilled syringes. Prior to agitation, GARDASIL 9 may appear as a clear liquid with a white precipitate. After thorough agitation, GARDASIL 9 is a white, cloudy liquid.

Pharmacological Properties:
HPV only infects human beings. Animal studies with analogous animal papillomaviruses suggest that the efficacy of L1 VLP vaccines may involve the development of humoral immune responses. Efficacy of GARDASIL 9 against anogenital diseases related to the vaccine HPV types in human beings is thought to be mediated by humoral immune responses induced by the vaccine, although the exact mechanism of protection is unknown.

Indications:
GARDASIL 9 is indicated in females and males from 9 years of age onward for the prevention of cervical, vulvar, vaginal, and anal cancer, precancerous or dysplastic lesions caused by HPV types 16, 18, 31, 33, 45, 52, and 58; genital warts (Condyloma acuminata) caused by specific HPV types; and persistent infections caused by HPV.

Dosage and Administration:
GARDASIL 9 should be administered intramuscularly as 3 separate 0.5-mL doses at 0, 2, and 6 months in the deltoid region of the upper arm or in the higher anterolateral area of the thigh. The second dose should be administered at least 1 month after the first dose, and the third dose should be administered at least 3 months after the second dose. All three doses should be given within a 1-year period. No studies have been performed to assess interchangeability for GARDASIL 9 and GARDASIL.
Active Ingredient:
Empagliflozin

Presentation:
Jardiance 10mg – Each film-coated tablet contains 10 mg of empagliflozin
Jardiance 25mg – Each film-coated tablet contains 25 mg of empagliflozin

Pharmacological Properties:
Empagliflozin is a reversible, highly potent (IC_{50} of 1.3 nmol) and selective competitive inhibitor of sodium-glucose co-transporter 2 (SGLT2). Empagliflozin does not inhibit other glucose transporters important for glucose transport into peripheral tissues and is 5000 times more selective for SGLT2 versus SGLT1, the major transporter responsible for glucose absorption in the gut. SGLT2 is highly expressed in the kidney, whereas expression in other tissues is absent or very low. It is responsible, as the predominant transporter, for the reabsorption of glucose from the glomerular filtrate back into the circulation. In patients with type 2 diabetes and hyperglycaemia a higher amount of glucose is filtered and reabsorbed.

Empagliflozin improves glycaemic control in patients with type 2 diabetes by reducing renal glucose reabsorption. The amount of glucose removed by the kidney through this glucuretic mechanism is dependent on blood glucose concentration and GFR. Inhibition of SGLT2 in patients with type 2 diabetes and hyperglycaemia leads to excess glucose excretion in the urine. In patients with type 2 diabetes, urinary glucose excretion increased immediately following the first dose of empagliflozin and is continuous over the 24 hour dosing interval. Increased urinary glucose excretion was maintained at the end of the 4-week treatment period, averaging approximately 78 g/day.

Increased urinary glucose excretion resulted in an immediate reduction in plasma glucose levels in patients with type 2 diabetes.

Empagliflozin improves both fasting and post-prandial plasma glucose levels. The mechanism of action of empagliflozin is independent of beta cell function and insulin pathway and this contributes to a low risk of hypoglycaemia. Improvement of surrogate markers of beta cell function including Homeostasis Model Assessment-β (HOMA-β) was noted. In addition, urinary glucose excretion triggers calorie loss, associated with body fat loss and body weight reduction. The glucosuria observed with empagliflozin is accompanied by mild diuresis which may contribute to sustained and moderate reduction of blood pressure.

Indications:
Jardiance is indicated in the treatment of type 2 diabetes mellitus to improve glycaemic control in adults as:

Monotherapy
When diet and exercise alone do not provide adequate glycaemic control in patients for whom use of metformin is considered inappropriate due to intolerance.

Add-on combination therapy
In combination with other glucose-lowering medicinal products including insulin, when these, together with diet and exercise, do not provide adequate glycaemic control.

Dosage & Administration:

Monotherapy and add-on combination
The recommended starting dose is 10 mg empagliflozin once daily for monotherapy and add-on combination therapy with other glucose-lowering medicinal products including insulin. In patients tolerating empagliflozin 10 mg once daily who have an eGFR ≥60 ml/min/1.73 m² and need tighter glycaemic control, the dose can be increased to 25 mg once daily. The maximum daily dose is 25 mg.

When empagliflozin is used in combination with a sulphonylurea or with insulin, a lower dose of the sulphonylurea or insulin may be considered to reduce the risk of hypoglycaemia.

Patients with renal impairment
Due to the mechanism of action, the efficacy of empagliflozin is dependent on renal function. No dose adjustment is required for patients with an eGFR ≥60 ml/min/1.73 m² or CrCl ≥60 ml/min.

Empagliflozin should not be initiated in patients with an eGFR <60 ml/min/1.73 m² or CrCl ≤60 ml/min. In patients tolerating empagliflozin whose eGFR falls persistently below 60 ml/min/1.73 m² or CrCl below 60 ml/min, the dose of empagliflozin should be adjusted to or maintained at 10 mg once daily. Empagliflozin should be discontinued when eGFR is persistently below 45 ml/min/1.73 m² or CrCl persistently below 45 ml/min.

Empagliflozin should not be used in patients with end stage renal disease (ESRD) or in patients on dialysis as it is not expected to be effective in these patients.

Patients with hepatic impairment
No dose adjustment is required for patients with hepatic impairment. Empagliflozin exposure is increased in patients with severe hepatic impairment. Therapeutic experience in patients with severe hepatic impairment is limited and therefore not recommended for use in this population.

Elderly patients
No dose adjustment is recommended based on age. In patients 75 years and older, an increased risk for volume depletion should be taken into account. In patients aged 85 years and older, initiation of empagliflozin therapy is not recommended due to the limited therapeutic experience.

Paediatric population
The safety and efficacy of empagliflozin in children and adolescents has not yet been established. No data are available.

Method of administration
The tablets can be taken with or without food, swallowed whole with water. If a dose is missed, it should be taken as soon as the patient remembers. A double dose should not be taken on the same day.
Contraindications:
Hypersensitivity to the active substance or to any of the excipients of the product.

Precautions:
Jardiance should not be used in patients with type 1 diabetes or for the treatment of diabetic ketoacidosis.
Use in patients with renal impairment
Jardiance should not be initiated in patients with an eGFR below 60 ml/min/1.73 m² or CrCl <60 ml/min. In patients tolerating empagliflozin whose eGFR is persistently below 60 ml/min/1.73 m² or CrCl <60 ml/min, the dose of empagliflozin should be adjusted to or maintained at 10 mg once daily.
Empagliflozin should be discontinued when eGFR is persistently below 60 ml/min/1.73 m² or CrCl <60 ml/min. In patients with ESRD or in patients on dialysis as it is not expected to be effective in these patients.
Monitoring of renal function
Due to the mechanism of action, the efficacy of empagliflozin is dependent on renal function. Therefore assessment of renal function is recommended as follows:
- Prior to empagliflozin initiation and periodically during treatment, i.e. at least yearly
- Prior to initiation of any concomitant medicinal product that may have a negative impact on renal function.
Hepatic injury
Cases of hepatic injury have been reported with empagliflozin in clinical trials. A causal relationship between empagliflozin and hepatic injury has not been established.
Elderly patients
The effect of empagliflozin on urinary glucose excretion is associated with osmotic diuresis, which could affect the hydration status. Patients aged 75 years and older may be at an increased risk of volume depletion. A higher number of these patients treated with empagliflozin had adverse reactions related to volume depletion as compared to placebo. Therapeutic experience in patients aged 85 years and older is limited, and there is no experience in clinical studies with empagliflozin in NYHA class III-IV.
Use in patients at risk for volume depletion
Based on the mode of action of SGLT-2 inhibitors, osmotic diuresis accompanying therapeutic glucosuria may lead to a modest decrease in blood pressure. Therefore, caution should be exercised in patients for whom an empagliflozin-induced drop in blood pressure could pose a risk, such as patients with known cardiovascular disease, patients on anti-hypertensive therapy with a history of hypotension or patients aged 75 years and older.
In case of conditions that may lead to fluid loss (e.g. gastrointestinal illness), careful monitoring of volume status (e.g. physical examination, blood pressure measurements, laboratory tests including haematocrit) and electrolytes is recommended for patients receiving empagliflozin. Temporary interruption of treatment with empagliflozin should be considered until the fluid loss is corrected.
Urinary tract infections
The overall frequency of urinary tract infection reported as adverse event was similar in patients treated with empagliflozin 25 mg and placebo and higher in patients treated with empagliflozin 10 mg. Complicated urinary tract infection (e.g. pyelonephritis or urosepsis) occurred at a similar frequency in patients treated with empagliflozin compared to placebo. However, temporary interruption of empagliflozin should be considered in patients with complicated urinary tract infections.
Cardiac failure
Experience in New York Heart Association (NYHA) class I-II is limited, and there is no experience in clinical studies with empagliflozin in NYHA class III-IV.
Urine laboratory assessments
Due to its mechanism of action, patients taking Jardiance will test positive for glucose in their urine.
Lactose
The tablets contain lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency, or glucose-galactose malabsorption should not take this medicinal product.

Drug Interactions:
Diuretics
Empagliflozin may add to the diuretic effect of thiazide and loop diuretics and may increase the risk of dehydration and hypotension.
Insulin and insulin secretagogues
Insulin and insulin secretagogues, such as sulphonylureas, may increase the risk of hypoglycaemia. Therefore, a lower dose of insulin or an insulin secretagogue may be required to reduce the risk of hypoglycaemia when used in combination with empagliflozin.
Effects of other medicinal products on empagliflozin
In vitro data suggest that the primary route of metabolism of empagliflozin in humans is glucuronidation by uridine 5'-diphosphoglucuronosyltransferases UGT1A3, UGT1A8, UGT1A9, and UGT2B7. Empagliflozin is a substrate of the human uptake transporters OAT3, OATP1B1, and OATP1B3, but not OAT1 and OCT2. Empagliflozin is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP).
Co-administration of empagliflozin with probenecid, an inhibitor of UGT enzymes and OAT3, resulted in a 26% increase in peak empagliflozin plasma concentrations (Cmax) and a 53% increase in area under the concentration-time curve (AUC). These changes were not considered to be clinically meaningful.
The effect of UGT induction on empagliflozin has not been studied. Co-medication with known inducers of UGT enzymes should be avoided due to a potential risk of decreased efficacy. An interaction study with gemfibrozil, an in vitro inhibitor of OAT3 and OATP1B1/1B3 transporters, showed that empagliflozin Cmax increased by 15% and AUC increased by
59% following co-administration. These changes were not considered to be clinically meaningful. Inhibition of OATP1B1/1B3 transporters by co-administration with rifampicin resulted in a 75% increase in $C_{max}$ and a 35% increase in AUC of empagliflozin. These changes were not considered to be clinically meaningful.

Empagliflozin exposure was similar with and without co-administration with verapamil, a P-gp inhibitor, indicating that inhibition of P-gp does not have any clinically relevant effect on empagliflozin.

Interaction studies conducted in healthy volunteers suggest that the pharmacokinetics of empagliflozin were not influenced by coadministration with metformin, glimepiride, pioglitazone, linagliptin, warfarin, verapamil, ramipril, simvastatin, torasemide and hydrochlorothiazide.

**Effects of empagliflozin on other medicinal products**

Based on *in vitro* studies, empagliflozin does not inhibit, inactivate, or induce CYP450 isoforms. Empagliflozin does not inhibit UGT1A1, UGT1A3, UGT1A8, UGT1A9, or UGT2B7. Drug-drug interactions involving the major CYP450 and UGT isoforms with empagliflozin and concomitantly administered substrates of these enzymes are therefore considered unlikely.

Empagliflozin does not inhibit P-gp at therapeutic doses. Based on *in vitro* studies, empagliflozin is considered unlikely to cause interactions with drugs that are P-gp substrates. Co-administration of digoxin, a P-gp substrate, with empagliflozin resulted in a 6% increase in AUC and 14% increase in $C_{max}$ of digoxin. These changes were not considered to be clinically meaningful.

Empagliflozin does not inhibit human uptake transporters such as OAT3, OATP1B1, and OATP1B3 *in vitro* at clinically relevant plasma concentrations and, as such, drug-drug interactions with substrates of these uptake transporters are considered unlikely.

Interaction studies conducted in healthy volunteers suggest that empagliflozin had no clinically relevant effect on the pharmacokinetics of metformin, glimepiride, pioglitazone, sitagliptin, linagliptin, simvastatin, warfarin, ramipril, digoxin, diuretics and oral contraceptives.

**Side Effects:**

**Minor hypoglycaemia**

The frequency of patients with minor hypoglycaemia was similar for empagliflozin and placebo as monotherapy, add-on to metformin, and add-on to pioglitazone with or without metformin. An increased frequency was noted when given as add-on to metformin and a sulfonylurea (empagliflozin 10 mg: 16.1%, empagliflozin 25 mg: 11.5%, placebo: 8.4%), and as add-on to insulin with or without metformin and with or without a sulphonylurea (empagliflozin 10 mg: 19.5%, empagliflozin 25 mg: 27.1%, placebo: 20.6% during initial 18 weeks treatment when insulin could not be adjusted; empagliflozin 10 mg: 36.1%, empagliflozin 25 mg: 34.8%, placebo 35.3% over the 78-week trial).

**Major hypoglycaemia (hypoglycaemia requiring assistance)**

No increase in major hypoglycaemia was observed with empagliflozin compared to placebo as monotherapy, add-on to metformin, add-on to metformin and a sulfonylurea, and add-on to pioglitazone with or without metformin. An increased frequency was noted when given as add-on to insulin with or without metformin and with or without a sulphonylurea (empagliflozin 10 mg: 0%, empagliflozin 25 mg: 1.3%, placebo: 0% during initial 18 weeks treatment when insulin could not be adjusted; empagliflozin 10 mg: 0%, empagliflozin 25 mg: 1.3%, placebo 0% over the 78-week trial).

**Vaginal moniliasis, vulvovaginitis, balanitis and other genital infection**

Vaginal moniliasis, vulvovaginitis, balanitis and other genital infections were reported more frequently in patients treated with empagliflozin compared to placebo, and the difference in frequency was less pronounced in males. The genital tract infections were mild or moderate in intensity.

**Increased urination**

Increased urination (including the predefined terms pollakiuria, polyuria, and nocturia) was observed at higher frequencies in patients treated with empagliflozin compared to placebo (3.4%, empagliflozin 25 mg: 3.2%) compared to placebo (1.0%). Increased urination was mostly mild or moderate in intensity. The frequency of reported nocturia was similar for placebo and empagliflozin (<1%).

**Urinary tract infection**

The overall frequency of urinary tract infection reported as adverse event was similar in patients treated with empagliflozin 25 mg and placebo (7.6%) and higher in empagliflozin 10 mg (9.3%). Similar to placebo, urinary tract infection was reported more frequently in patients with a history of chronic or recurrent urinary tract infections. The intensity (mild, moderate, severe) of urinary tract infection was similar in patients treated with empagliflozin and placebo. Urinary tract infection was reported more frequently in females treated with empagliflozin compared to placebo; there was no difference in males.

**Forensic Classification:**

P1S1S3
From the largest head-to-head study, ANORO® Ellipta® doubled the lung function improvement versus tiotropium among COPD patients

Umededimus 62.5 mcg / Vilanterol 25 mcg
One Inhalation, Once Daily

ANORO® ELLIPTA®
Umededimus/vilanterol
breathe...

Notes to Prescriber
- Patients should not stop therapy with Anoro in COPD without physician supervision
- Anoro should not be used in patients with asthma and it is not indicated for treatment of acute episodes of bronchospasm

**ANORO® Ellipta® Inhalation Powder, Pre-dispensed 62.5 mcg / 25 mcg (62.5 mcg umededimus and 25 mcg vilanterol)**

**Active Ingredients**
- Umededimus: 62.5 mcg
- Vilanterol: 25 mcg

**Indications**
- For maintenance treatment of chronic obstructive pulmonary disease (COPD) in patients with chronic obstructive pulmonary disease (COPD) who require long-term maintenance treatment according to guidelines
- For the treatment of chronic obstructive pulmonary disease (COPD) in patients who require long-term maintenance treatment according to guidelines

**Contraindications**
- Hypersensitivity to the active substances or any of the excipients
- Warnings and Precautions
  - Should not be used in patients with asthma
  - In patients with asthma, Anoro should not be used in patients with asthma and it is not indicated for treatment of acute episodes of bronchospasm
  - The dose should be reduced in patients with moderate or severe renal impairment

**Precautions for Use**
- In patients with asthma, Anoro should not be used in patients with asthma and it is not indicated for treatment of acute episodes of bronchospasm
- In patients with COPD, Anoro should not be used in patients with asthma and it is not indicated for treatment of acute episodes of bronchospasm

**ADVERSE REACTIONS**
- The following adverse effects have been reported with a frequency of common (>1/100) and (<1/10):
  - Urinary tract infections, sinusitis, rhinitis, pharyngitis, cough, chest pain, dyspepsia, abdominal pain, constipation, dry mouth

**Product Information**
- Umededimus/vilanterol (ANORO® Ellipta®)
- For further information, please see the Summary of Product Characteristics (SmPC) and the Summary of Product Characteristics (SmPC)

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