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Editorial note:

Traditional Chinese Medicine (TCM) has been used for a long time in Hong Kong and China. There are misconceptions that TCM is not harmful as natural products are involved. In addition, large variation in clinical response to a standard dose of medicine was also noted in individuals with certain genetic makeup. Dr WT Poon has provided a review of the important TCM poisonings as well as difference in genetic makeup causing altered blood level, and hence clinical response to drugs. We welcome any feedback or suggestions. Please direct them to Dr. Janice Lo (e-mail: janicelo@dh.gov.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Laboratory Role in Toxicology: From Diagnostic to Theranostic

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Introduction

Toxicology analysis involves detection, identification and measurement of foreign compounds and their metabolites in biological and other specimens. It plays a useful role in the management of poisoned patients when the diagnosis is in doubt, the administration of antidotes or protective agents is contemplated, or the use of active elimination therapy is being considered. As the scope and complexity of clinical toxicology continues to increase, continuing effort is required for the laboratory to expand its diagnostic capability and coverage. Apart from patient care, identification of a lethal or emerging toxin also serves to provide useful

information for toxico-vigilance of potential public health threats and helps to prevent further poisonings. Some common and important herbal poisonings that have occurred in Hong Kong would be discussed as examples.

Apart from poisoning diagnosis, laboratory test can be used to predict the risk of adverse event to drugs in individual patients. It is now feasible to identify the genetic basis for certain toxic side effects and drugs will then be prescribed only to those who are not genetically at risk. Theranostic is the term used to describe the process of diagnostic therapy for individual patients - to test them for possible reaction to taking a new medication and to tailor a treatment for them

based on the test results. In Hong Kong, genotyping for human lymphocyte HLA-B*1502 is recommended prior to administering carbamazepine for patients in order to avoid the development of Stevens-Johnson syndrome. An increasing number of pharmacogenetic tests are now available for clinical application. The criteria required of a pharmacogenetic test to make it useful for local application would be discussed.

Laboratory Role in Herbal Poisonings

Chinese herbal remedies have a history that dates back more than 5,000 years ago. The discovery of herbal remedies is ascribed to legendary emperor Shen Nung who was noted for tasting hundreds of herbs and said to have died of a toxic overdose. As is the case with western pharmaceuticals, some herbs are toxic and must be used with caution. Herbal poisonings often occur as a result of overdose or erroneous substitution. There are different ways to name an herb: the common name, the Latinized pharmaceutical name and the scientific name. Common names can be very loose. The same name can be applied to several herbs and the same herb can have several names. This frequently leads to erroneous substitution of one herb by a toxic counterpart and result in poisonings. Toxicology analysis can help to identify culprit toxin in unused herbs, herbal remnants or biological samples and confirm a diagnosis of poisoning.

Aristolochic acid nephropathy

Aristolochic acid nephropathy (AAN) is a unique nephropathy characterized by rapidly progressive interstitial fibrosis and urothelial cancer. It is related to the prolonged intake of Chinese herbal remedies containing the nephrotoxic and carcinogenic aristolochic acid 馬兜鈴酸 (AA). AAN was recognized and reported in the Chinese and English literature in the early 1960s. In 1963, Peters and Hedwall observed the loss of concentrating ability of the kidney as a result of intoxication with AA. However, there has been a delay in the appreciation of these findings until the early 1990s. In 1993, a Belgian weight loss clinic incorrectly administered Aristolochiae Fang chi (廣防己) in its slimming regimen. More than

100 patients subsequently developed severe renal failure.[1]

While AAN has been reported in many countries, there was no local case reported in Hong Kong until 2004. The first case presented to hospital with progressive renal failure and bladder cancer after taking herbs for 6 months. The herbs were sent for toxicology analysis and AA was detected. The non-toxic Herba Solani Lyrati (白英) in patient's TCM formula was found to be mixed up with the AA-containing Herba Aristolochia Mollissemiae (尋骨風). Both herbs are furry in appearance and share a common name (白毛藤). The erroneous substitution was found to have occurred at the wholesaler level for many years. This led to the subsequent discovery of more AAN cases and total ban of AA-containing herbs in Hong Kong. AAN is a terrible example of what can go wrong when quality control measures of herbal products are insufficient or not observed. It also highlights the importance of improving the nomenclature system of herbs.[2]

Hidden Aconite Poisoning

Aconite poisoning is one of the most common causes of herbal poisonings in Hong Kong. Both Radix Aconiti (川烏) and Radix Aconiti Kusnezoffii (草烏) have been used in Traditional Chinese Medicine for the treatment of various musculoskeletal disorders. Aconitum alkaloids are the active ingredients and the source of toxicity. Toxic symptoms include numbness, weakness, cardiac arrhythmia and hypotension. Patients with severe poisoning may die from refractory ventricular arrhythmias.

There are cases of aconite poisoning in which no aconite herb was present in the TCM prescription. Yet aconitum alkaloids were detected in the urine specimens and leftover herbal broth. Aconite was thus considered the 'hidden' cause of their acute poisoning. Inadvertent contamination with an aconite herb is a possible explanation. Intuitively, a mix-up between aconite herbs and other herbs can occur at a number of stages, including harvesting, during processing, transportation, storage, and dispensing. The fresh aconite roots

are extremely toxic and must be processed before use. In addition, processed aconite roots should be decocted (先煎) for one or two hours in advance of other herbs to further reduce the toxicity. Naturally this particular procedure will not be carried out if the presence of an aconite herb is not intended. 'Hidden' aconite poisoning, hence, is far more dangerous than intentional use of aconite. It highlights the importance of quality assurance in herbs with low margins of safety.[3]

Generally, herbal poisoning is difficult to diagnose. This is in part due to inadequate knowledge about the toxicity of Chinese medicine.[4] In Taiwan it was reported that 47% of the potentially toxic effects of Chinese traditional medicines were either unknown or could not be found in the literature. Hence, toxicological problems associated with the use of herbal medicines may not be readily recognised. For example, yunaconitine was identified in some poisonings caused by aconitum species of Yunnan origin. It is not one of the common toxins (aconitine, hyaconitine, and mesaconitine) seen in aconite poisoning. The diagnosis would have been missed in these cases if laboratory screening for yunaconitine was not included.[5] While routine comprehensive toxicology screen can cover the commonly used western drugs, they are not sufficient for herbal poisoning. The herbal matrix is extremely complex and target analysis based on LC-MS/MS method, with enhanced sensitivity and specificity, is often required for laboratory diagnosis.

Laboratory Role in Theranostics

The second part of this article will be focused on prevention of drug toxicity. While standard doses of most medicines work well for most people, some cause annoying and sometimes dangerous side effects. Although most severe adverse reactions are due to errors in prescription, allergies, or interactions between several medicines, occasionally toxic side effects may be explained by genetic variability which affects an individual's response to drug therapy. If we can identify the genetic basis for certain toxic side effects, drugs could be prescribed only to those who are not genetically at risk. For example,

enzymes belonging to cytochrome P450 (CYP450) superfamily take part in metabolizing drugs. People can be classified into groups of poor, intermediate, extensive and ultra-rapid metabolizers. The poor metabolizers are at increased risk of drug-induced side effects due to diminished drug elimination or lack of therapeutic effect resulting from failure to generate the active form of the drug. On the other hand, the ultra-rapid metabolizers have increased metabolic capacity and may require an increased dosage.

An increasing number of pharmacogenetic tests are now available for clinical application. The US FDA has categorized them into 'for information only', 'recommended', and 'required'. On the other hand, polymorphic alleles of drug response-related genes vary among ethnic groups and overseas guidelines may not be applicable. Before applying a pharmacogenetic test to local practice, one should consider the following questions [6]: How prevalent is the genotype of interest? How closely is the polymorphism linked to a consistent phenotypic drug response? Are there metabolic, environmental or other significant influences on drug response? How effective are current monitoring strategies for preventing severe adverse drug reactions and predicting drug response? What are the sensitivity and specificity of the genomic test? How does the genomic test alter these outcomes? What alternative therapeutic options are available? Two examples are discussed below as models for local application of pharmacogenetic testing.

Carbamazepine induced Stevens - Johnson syndrome

Carbamazepine (CBZ) is a commonly prescribed drug for treatment of epilepsy, bipolar disorder and neuropathic pain. However, CBZ is also associated with hypersensitivity reactions that range from benign urticaria to life-threatening cutaneous disorders, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The latter two disorders carry significant mortality even with early diagnosis and prompt withdrawal of causative drugs. In the past, there is no test or biomarker that can predict carbamazepine-induced cutaneous adverse

reactions. Nowadays, it is known that SJS/TEN caused by CBZ is strongly associated with the HLA-B*1502 gene. The allele is highly prevalent in Han Chinese with a carrier rate of ~10%. One Chinese study found that an individual with this genotype had ~1400-fold higher risk for development of SJS than an individual with the wild-type.[7] HLA-B*1502 is therefore an ideal marker to predict CBZ-induced SJS with high sensitivity and specificity. Since 2008, the Hospital Authority has issued an alert to advise all clinical doctors to test for the HLA-B*1502 allele in new patients receiving this drug and to prescribe carbamazepine only if tested negative. Patients who carry HLA-B*1502 allele would be offered alternative treatment options.

Thiopurine methyltransferase genotyping for azathioprine toxicity

Azathioprine and other mercaptopurine agents are used in gastrointestinal inflammatory disorders and leukemia. These compounds are metabolized to inactive metabolites by several metabolic pathways. One major pathway involves the thiopurine methyl transferase (TPMT) enzyme. Patients with TPMT deficiency may develop life-threatening myelosuppression with standard doses of azathioprine. TPMT genotyping is recommended by US FDA as a useful adjunct to a regimen for prescribing azathioprine. The TPMT*3A mutant allele predominates in Caucasians and shows zero activity. Caucasians show a trimodal distribution of TPMT activity, with 89-94% possessing normal enzyme activity, 6-11% intermediate activity due to heterozygosity, and 0.33% extremely low or absent activity due to homozygosity.[8] The latter group would require substantial dose reduction. On the other hand, the role of genotyping in Han Chinese has not been established. Han Chinese shows a unimodal distribution of TPMT activity. The TPMT*3A mutant allele has not been detected in Chinese. Instead TPMT*3C is the predominate mutant allele and shows moderate activity.[9] Extremely low or absent activity is therefore very rare in Chinese. Besides, one third of Chinese patients with intermediate activity did not have any TPMT mutant alleles detected.[10] For pre-treatment

testing purpose, TPMT enzyme level may be more useful than genotyping.

Concluding remarks

Paracelsus (1493-1541) is considered to be the father of toxicology. He is credited with the classic toxicology maxim, "All things are poison and nothing is without poison" All substances are toxic under the right conditions. It is widely accepted that dosage is the chief criterion regarding the toxicity of a chemical. However, with the advances in knowledge and applications of personalized medicine, it is expected that host factor would become another major criterion for the assessment of drug toxicity in future.

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