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Of Potions, Poisons, Polygonum and Pre-emptive Polymorphism

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Abbreviations: CYP: cytochrome P450; DILI: drug-induced liver injury; PM: P*olygonum multiflorum*; HLA: Human leukocyte antigen; ALT: alanine transaminase; MHC: Major histocompatibility complex; CD8+: Cytotoxic T lymphocytes; *PTPN22*: protein tyrosine phosphatase non-receptor type 22 gene; Lyp: lymphoid protein tyrosine phosphatase.

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Oberon:

Fetch me that flower. The herb I show'd thee once.

The juice of it on sleeping eyelids laid

Will make man or woman madly dote

Upon the next live creature that it sees.

Fetch me this herb,

Act 2, Scene 1: A Midsummer Night's Dream

There are scores of references to plants and herbs in William Shakespeare's plays; he was obviously very knowledgeable about their perceived effects. The secret to the highly potent love potion Oberon asks Puck to find, lies in the purple, yellow and white flower, 'love-in-idleness', a folk name for the wild pansy (*Viola tricolour*). When scientists from Royal Society of Chemistry and Quest International put this to the test 400 years later, they concluded that 'Wild pansies were noted in herbal folklore medicine. It was attributed with many properties, but, falling in love was probably the invention of Shakespeare' [1]. Master craftsman had woven a myth into the plot with dramatic effect.

Over the generations, herbal remedies have remained popular, their status sustained more often by belief than evidence supporting efficacy. The output of 'Chinese Materia Medica' amounted to US$83.1 billion in 2012, the market growing by over 20% from the previous year [2]. As herbs are 'natural', formulations derived from them are deemed safe; there is neither coherence in categorisation of these products nor consistency in the regulatory requirements and processes internationally [3]. Irony is that irrespective of lack of robust evaluation of their efficacy, adverse hepatic reactions attributed to herbs are being reported increasingly. In a study involving 25,927 cases of drug-induced liver injury (DILI) from mainland China, herbal and dietary supplements were the most common group of causal agents accounting for 27% of these cases [4]. Another systematic review identified P*olygonum multiflorum* (PM) as the most common causative agent associated with herb-induced liver injury [5].

Source of ambiguity in the properties of potions and poisons, effects of herbs and medicine is perhaps in the origin of species! When life expanded from sea and took to land, plants anchored themselves, setting roots while the animals roamed. Plants relied upon animals to disperse pollens and seeds, but, needed to protect themselves from mobile predators. The conflict that arose 300-500 million years ago led to the emergence of defensive, toxic chemicals in plants and accelerated the evolution of cytochrome P450 (CYP) families I-IV to detoxify plant metabolites in herbivorous animals first. It is estimated that at least 25% of all modern medications are derived from plants and many of these are metabolized by CYPs. Hence, it is unsurprising if similar pathogenic mechanisms underlie drug- and herb-induced liver injury.

In this issue of Hepatology, Chaopeng Li and colleagues report findings of their investigations into histogenetic risk factors associated with PM-DILI [6]. Initially, 27 gene loci within the Human leukocyte antigen (HLA) region were sequenced in 11 PM-DILI patients and allele frequencies were compared with that of 10,689 Han Chinese included in a population based database to identify an association of *HLA-B*35:01* with liver injury. This discovery was then validated in an independent group of 15 PM-DILI cases, 33 cases of DILI due to other medications and 99 population based controls. In a further prospective cohort study, 3/8 (37.5%) of *HLA-B*35:01* carriers and 3/64 (4.7%) non-carriers developed 2-fold elevation of alanine transaminase (ALT) confirming that individuals carrying *HLA-B*35:01* are at a higher risk of developing DILI when treated with herbal remedies containing PM.

These studies followed good examples of translational research; discovery phase included well characterised cases (admittedly a small number) of DILI due to a single agent (*Polygonum multiflorum*). A combination of healthy and disease controls, from the general population as well as hospital-base was used to validate the association of *HLA-B*35:01* with PM-DILI. Potential application of *HLA-B*35:01* testing was piloted in a prospective cohort to assess test's performance characteristics. By design, the study involved one ethnic group and focused on histogenetic factors only, therefore, the results have to be viewed within such a pre-defined context. Beyond these specifics, identification of MHC class 1 allele as a risk factor for PM-DILI indicates that adaptive immune response is a key step in the pathogenesis of liver injury in susceptible individuals (Figure 1).

Liver is central to the biotransformation of a vast majority of herbal compounds as it is for drugs; the process generally leads to the formation of stable metabolite and their safe excretion. Genetic and environmental factors that influence the expression and activities of

the drug metabolising enzymes (phase I and II), transporters involved in the excretion (phase III) of these metabolites determine the rate of formation and accumulation of reactive metabolites. Oxidative stress induced by reactive metabolites or their covalent binding to cellular proteins interfering with vital cellular function can lead to hepatocellular injury particularly when cellular defense is impaired. Support to this hypothesis comes from the observations that furano diterpenoids in Germander (*Teucrium chamaedrys*) are oxidized by CYP3A into reactive metabolites and pyrrole-protein adducts have been detected in a patient with hepatic sinusoidal occlusion syndrome secondary to Chinese medicine 'Tusanqi' containing '*Gynura segetum*'. Further, those who drink germander tea for a long period of time have anti-microsomal epoxide hydrolase autoantibodies in their sera. Therefore, these herb specific events may form essential steps in the pathogenesis, but, not always sufficient to generate clinically significant DILI.

HLA molecules are central to the activation of T-cells, responsible for initiating the inappropriate immune response that underlies DILI; both branches of the highly specific adaptive immune response rely on the selective presentation of antigens to T-cells by HLAs, highly polymorphic proteins also known as major histocompatibility complex (MHC) proteins. MHC class I molecules are expressed by almost all nucleated cells including hepatocytes. MHC class I proteins usually associate with peptide antigens generated by the partial degradation of self-proteins which could include metabolite-cellular protein adducts generated by a herbal compound. The MHC I-antigen complex is then expressed on the cell surface and elicits an immune response if a non-self-antigen is recognised, causing the activation of CD8+ T-cells, which leads to the cell mediated killing of the original cell (Figure 1). Recently, a polymorphism in the protein tyrosine phosphatase non-receptor type 22 gene (*PTPN22*) coding for lymphoid protein tyrosine phosphatase (Lyp) has been associated

with DILI caused by multiple drugs [7]. Switch in function associated with variant allele reduces immune tolerance of T-cells promoting auto-immunity. Interestingly, specific minor *PTPN22* allele is virtually absent in Asian and African populations.

HLA alleles have been shown to identify the risk of DILI from over 15 currently used medications [8] and now a herb has joined the list; time has come to use HLA genotyping as a part of our diagnostic armamentarium. Effective prevention of DILI using genetic markers would be the ultimate goal of pharmacogenetics and has been put forward in relation to lumiracoxib in the past [10]. As with lumiracoxib, the effectiveness of such an approach relies upon the added value a particular compound brings to the existing therapeutics. As authors propose, if pre-prescription screening for *HLA-B*35:01* were to be performed in 72 individuals, 50% (3/6) PM-DILI (based on doubling of ALT) would be prevented and 7% (5/72) of others carrying *HLA-B*35:01* (false positive) would not be offered herbal remedy. However, such arguments must be tempered with caution as important evidence for health benefits from a number of herbs such as P*olygonum multiflorum* is lacking. That is a metaphorical green elephant in the room!

Figure legend:

Figure 1: Putative mechanisms underlying the pathogenesis of liver injury due to drugs and herbs. Agent (drug/ herb) specific factors influence the proximal events and distal events are determined by host (genetic) factors.

Foot notes: HLA: Human Leukocyte Antigen; MHC: Major Histocompatibility Complex, CD8+: Cytotoxic T lymphocytes; *PTPN22*: protein tyrosine phosphatase non-receptor type 22 gene; Lyp: lymphoid protein tyrosine phosphatase.

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