



## Drug-induced liver injury: Is it somehow foreseeable?

Giovanni Tarantino, Matteo Nicola Dario Di Minno, Domenico Capone

Giovanni Tarantino, Matteo Nicola Dario Di Minno, Department of Clinical and Experimental Medicine, Section of Hepatology in Internal Medicine, Federico II University, Medical School of Naples, 5 80131 Napoli, Italy

Domenico Capone, Department of Neurosciences, Unit of Clinical Pharmacology, Federico II University, Medical School of Naples, 5 80131 Napoli, Italy

**Author contributions:** All the authors equally contributed towards writing and editing the manuscript; All authors approved the final version of the manuscript.

**Correspondence to:** Giovanni Tarantino, MD, Professor, Department of Clinical and Experimental Medicine, Section of Hepatology in Internal Medicine, Federico II University, Medical School of Naples, Via S. Pansini, 5 80131 Napoli, Italy. [tarantin@unina.it](mailto:tarantin@unina.it)

Telephone: +39-81-7462024 Fax: +39-81-5466152

Received: April 22, 2009 Revised: May 13, 2009

Accepted: May 20, 2009

Published online: June 21, 2009

15(23): 2817-2833 Available from: URL: <http://www.wjgnet.com/1007-9327/15/2817.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.2817>

### INTRODUCTION

Drug metabolism is the major determinant of drug clearance and the factor most frequently responsible for inter-individual differences in drug pharmacokinetics. Most adverse hepatic reactions require metabolism of the drug to form reactive metabolites and free radicals (indirect toxicity), that subsequently lead to fatal insults, sensitization to the lethal effects of the innate immune system, or haptenization eliciting an immunoallergic response of the adaptive immune system. Besides licensed drugs, herbal and natural supplements are recognized as causing hepatotoxicity with increasing frequency as patients turn more and more to alternative medicine<sup>[1]</sup>.

Many environmental and developmental factors can interact with each other and with genetic factors to affect drug response and the metabolic activation or inactivation of drugs generally used in medical practice.

Oxidation, reduction and hydrolysis are the main pathways along drug metabolism before excretion. The most important enzyme system of phase I metabolism is the cytochrome P-450 (CYP) system, a microsomal superfamily of isozymes that catalyze the oxidation of many drugs. The electrons are supplied by NADPH/CYP reductase, a flavoprotein that transfers electrons from NADPH (the reduced form of nicotinamideadenine dinucleotide phosphate) to CYP. Multiple forms of CYP enzymes play important roles in the oxidation of structurally diverse xenobiotics. CYP3A (about 30% of total CYP) and CYP2C (about 20% of total CYP) enzymes are major forms. Human cytochrome CYP3A subfamily members (mainly CYP3A4) mediate the metabolism of many marketed drugs (amiodarone, amlodipine, clarithromycin, cyclosporine, erythromycin, lovastatin, nifedipine, tamoxifen, terfenadine, verapamil, R-warfarin) and thus play a critical role in drug metabolism. Furthermore, P-glycoprotein and CYP3A are frequently co-expressed in the same cells and share a large number of substrates and modulators<sup>[2]</sup>.

Other important human drug-metabolizing enzymes are CYP1A2 (caffeine, estradiol, lidocaine, tacrine, theophylline, verapamil, R-warfarin), CYP2C9 (diclofenac, phenytoin, piroxicam, tetrahydrocannabinol, tolbuta-

### Abstract

The classic view on the pathogenesis of drug-induced liver injury is that the so-called parent compounds are made hepatotoxic by metabolism (formation of neo-substances that react abnormally), mainly by cytochromes P-450 (CYP), with further pathways, such as mitochondrial dysfunction and apoptosis, also playing a role. Risk factors for drug-induced liver injury include concomitant hepatic diseases, age and genetic polymorphisms of CYP. However, some susceptibility can today be predicted before drug administration, working on the common substrate, by phenotyping and genotyping studies and by taking in consideration patients' health status. Physicians should always think of this adverse effect in the absence of other clear hepatic disease. Ethical and legal problems towards operators in the health care system are always matters to consider.

© 2009 The WJG Press and Baishideng. All rights reserved.

**Key words:** Drug-induced liver injury; Cytochrome P-450; Drug metabolism; Pharmacogenomics; Herbal remedies

**Peer reviewer:** Dr. Yukihiro Shimizu, Kyoto Katsura Hospital, 17 Yamada-Hirao, Nishikyo, Kyoto 615-8256, Japan

Tarantino G, Di Minno MND, Capone D. Drug-induced liver injury: Is it somehow foreseeable? *World J Gastroenterol* 2009;

mide, S-warfarin), CYP2C19 (diazepam, hexobarbital, S-mephenytoin, omeprazole, pentamidine, propranolol, R-warfarin), and CYP2D6 (codeine, debrisoquine, dextromethorphan, encainide, haloperidol, metoprolol, mexiletine, paroxetine, phenothiazines, propranolol, risperidone, sertraline, tricyclic antidepressants, venlafaxine) as highlighted by commonly used probe cocktails<sup>[3,4]</sup>. The expression of drug metabolizing enzymes, mainly CYP, shows significant interspecies differences and variability among human individuals (polymorphic or inducible enzymes) which makes the accurate prediction of the metabolism of various compounds in humans difficult. For example, patients who metabolize certain drugs rapidly may require higher, more frequent doses to achieve therapeutic concentrations; patients who metabolize certain drugs slowly may need lower, less frequent doses to avoid toxicity, particularly for drugs with a narrow interval of safety.

## HEPATOTOXICITY: GENERAL CONCEPTS

Several key issues need to be addressed to study drug induced liver injury (DILI), that is, what metabolites will be formed (metabolic profile); which enzymes are involved and to what extent; whether drug metabolism will be affected directly (drug-drug interactions) or indirectly (enzyme induction) by the administered compound. Drug metabolism studies are routinely performed in laboratory animals, but they are not sufficiently accurate to predict the metabolic profiles of drugs in humans. In fact, hepatotoxicity due to idiosyncratic reactions cannot be detected by conventional animal toxicity studies. Furthermore, predisposing factors in humans such as ethanol-induced metabolic sensitivity to acetaminophen hepatotoxicity do not exist in the animal model. Interspecies differences in bioavailability, distribution and metabolism may also explain a number of false positives and false negatives. There is clearly a need to better understand the drug metabolism enzyme profile of the most commonly used non-rodent species, i.e. the dog and the monkey. Some false negative results may be related to insufficient (not in the pharmaceutical industry) exposure of the animals either because the doses tested were too low or because intestinal absorption was poor. Many of these issues can now be addressed by the use of relevant human *in vitro* models, which may speed up the understanding of drug toxicity. Human hepatocytes are the closest *in vitro* model to the human liver and they are one of the few models which can produce a metabolic profile of a drug which is very similar to that found *in vivo*. However, the use of human hepatocytes is restricted, because limited access to suitable tissue samples prevents their use in high-throughput chemical and genetic screens<sup>[5]</sup>. Comparative studies on liver microsomes and cells from animal species, including humans, are very useful for demonstrating species differences in the metabolic profile of a given drug and are of great value in the selection of animal species for later pharmacokinetic and toxicological studies<sup>[6]</sup>.

CYP-engineered cells (or microsomes from CYP-engineered cells, for example, Supersomes) have made the

identification of CYPs involved in the metabolism of a drug candidate more straightforward and much easier<sup>[7]</sup>. However, the screening of substances acting as potential CYP inducers can be conducted only in cellular systems fully capable of transcribing and translating CYP genes.

## EPIDEMIOLOGY AND CLINICAL ASPECTS OF DRUG INDUCED LIVER INJURY

Drug hepatotoxicity has been evaluated in case histories, surveys based on retrospective record reviews, and spontaneous adverse drug reactions reported to national pharmacovigilance systems, but in relatively few epidemiologic studies. Approximately 1 in 100 patients develops DILI during hospitalisation. DILI is frequently missed and, therefore, DILI detection by diagnosis will result in misleadingly low incidence rates<sup>[8]</sup>. Unfortunately, patients with drug-induced hepatocellular jaundice have an 11.7% chance of progressing to death or transplantation<sup>[9]</sup>.

DILI cases have been reported to constitute approximately 6% of all out-patients and 3% of referrals and to occur more often in women<sup>[10]</sup>. The incidence of drug-induced hepatitis is higher in patients over 40 years of age<sup>[11]</sup>. Acute liver failure or injury not clearly attributable to other known causes occurred in the order of 1 per 10 000 person-years among diabetic patients treated with oral hypoglycemic drugs or insulin<sup>[12]</sup>.

The long term outcome of drug-related liver disease is unknown. To study the natural history of histologically proved drug-induced hepatotoxicity, 44 patients with liver biopsies coded as drug-related liver disease were identified from hospital records. Initial histology showed acute hepatitis in 6, chronic hepatitis in 20, and cholestasis in 18. At a median of 5 years follow-up, one third of patients had persistent significant abnormalities in their liver blood tests. Factors predicting persistence or development of chronic liver disease were fibrosis and continued exposure to the drug<sup>[13]</sup>. To identify and quantify the risk of acute liver injury associated with individual drugs, authors reviewed and integrated all the published epidemiologic research on the subject. Participants were selected according to their use of selected agents [nonsteroidal antiinflammatory drugs (NSAIDs), antibiotics, acid-suppressing drugs, other drugs suspected of being hepatotoxic] during the study period. Among the agents, authors found a group of important hepatotoxic drugs, including chlorpromazine and isoniazid, with an associated incidence rate of acute liver injury greater than 100/100 000 users. Agents with less risk but greater than 10/100 000 users were amoxicillin-clavulanic acid and cimetidine<sup>[14]</sup>.

DILI with an incidence rate of near 1 per 100 000 encloses a spectrum of clinical disease ranging from no symptoms with mild biochemical abnormalities to fatal, fulminant hepatitis. The majority of adverse liver reactions are idiosyncratic in nature; in fact, about 10% of all acute liver failure cases are attributed to this type of reaction. They can occur in some instances up to three months after the causative medication was last taken.

The diagnosis of DILI is prevalent clinically, and based primarily on history, that is, exclusion of other hepatic diseases (hepatitis A, B, C, Epstein-Barr virus, cytomegalovirus, ischemia, and biliary tract disease), high likelihood of suspicion based on a strict cause-effect sequence, the duration of latency to symptomatic presentation, the presence of immune-mediated hypersensitivity (hypereosinophilia, fever and rash) and the response to drug withdrawal. Re-challenge is not advised in cases with a hypersensitivity basis, although this is the most definitive means of diagnosis. Some of the hypersensitivity cases are associated with autoantibodies to CYP, which can be used to confirm the diagnosis; for example, halothane is associated with anti-CYP2E1, anticonvulsants are associated with CYP3A4, dihydralazine hepatitis is associated with anti-CYP1A2, and tienilic acid (a diuretic drug withdrawn from the market because of hepatic failure) is associated with anti-CYP2C9. In the cases of metabolic idiosyncrasy, careful reintroduction of the offending drug may be accomplished without recurrence of the liver disease but should be done only when the drug is absolutely necessary and with careful monitoring. In some instances, liver biopsy can be of help, showing characteristic features, but it usually is not necessary and tells more about prognosis than etiology.

DILI can be of hepatocellular (increase of both the transaminases, alanine-aminotransferase/aspartate-aminotransferase), cholestatic (predominant rise in serum alkaline phosphatase and/or  $\gamma$ -glutamyl-transferase, GGT) or mixed type. Negative prognostic factors are an elevated serum bilirubin level and high ammonia levels with a mortality of approximately 10%<sup>[15]</sup>. A further less favourable index is a marked hypoprothrombinemia. Overall, chronic liver injury may occur in up to 5%-6% of the patients on some drugs, even though the putative offending substance is withdrawn<sup>[16]</sup>.

## LIVER HISTOLOGY

As previously emphasized, the pathogenesis of drug- or toxin- induced liver injury usually involves the participation of "toxic metabolites" that either elicit an immune response or directly affect the biochemical processes or functions of the cell<sup>[17]</sup>. Although the same basic process determines the DILI appearance, the histopathological liver changes in these cases vary, including: (a) necrosis, which commonly occurs in acinar zone 3, (b) abundant neutrophil and/or eosinophil infiltration, (c) hepatocytic and/or canalicular cholestasis with little or no inflammation, (d) microvesicular steatosis mixed with macrovesicular steatosis, and (e) presentation of epithelioid cell granuloma. There are no significant differences in liver histopathology between acute and chronic DILI groups, except that the fibrosis and the ductular proliferation are different.

## DAMAGE MECHANISMS

Drug metabolites can be free radicals or electrophilic chemicals that undergo or promote a variety of chemical

reactions, such as the depletion of reduced glutathione; covalent binding to proteins, lipids, or nucleic acids; or induction of lipid peroxidation. All of these have consequent direct effects on cellular organelles such as mitochondria, the endoplasmic reticulum, the cytoskeleton, microtubules, or on the nucleus. They may also indirectly influence cellular structures through the activation and inhibition of signaling kinases, transcription factors, and gene-expression profiles. The subsequent intracellular stress leads to cell death caused by either cell shrinkage and nuclear disassembly (apoptosis) or swelling and lysis (necrosis). Liver injury is characterized by hepatocyte death; that is the main event, although bile duct epithelium or sinusoidal endothelial cells may also be involved.

### **CYP-mediated Biotransformation: the common substrate**

While several CYPs are involved in the synthesis of bile acids and steroid hormones and the metabolism of fatty acids, retinoic acid, prostaglandins and eicosanoids, a limited number of CYPs (15 in humans) are primarily involved in xenobiotic metabolism. The xenobiotic-metabolizing CYPs are found in families 1-4. Since a single CYP can metabolize a large number of structurally diverse compounds these enzymes can collectively metabolize chemicals found in the diet, environment and administered as drugs. While metabolism of xenobiotics such as drugs is required to efficiently eliminate them from the body, as noted earlier, certain chemicals are metabolically activated to reactive derivatives that cause cell toxicity and cancer. Among these, the CYPs that metabolically activate toxicants and carcinogens are limited to some forms including CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2E1, and to a limited extent the CYP3A subfamily.

### **ATP depletion**

Acetaminophen overdose causes liver injury by mechanisms involving glutathione depletion, oxidative stress and mitochondrial dysfunction. Acetaminophen-induced decreases in mitochondrial reduced glutathione and ATP content, and cytosolic leakage of cytochrome c are attenuated by cyclosporin A, suggesting that mitochondrial oxidative stress and ATP depletion resulting from mitochondrial permeability transition (MPT) are the principal mechanisms involved in acetaminophen-induced liver injury<sup>[18]</sup>.

The role of ATP-depletion-dependent necrosis has further been ascertained. Recently, using TUNEL labeling and caspase 3 activation, it was observed that acetaminophen induced the MPT and ATP-depletion-dependent necrosis or caspase-dependent apoptosis as determined, in part, by ATP availability from glycolysis<sup>[19]</sup>.

### **Binding to nuclear or cytoplasmic constituents**

Inducers can increase the bioactivation of drugs that contribute to hepatotoxicity *via* reactive intermediates. Nuclear receptors are key mediators of drug-induced changes in the expression of drug clearance pathways. However, species differences in nuclear receptor activation make the

prediction of CYP induction in humans from data derived from animal models problematic. Drugs have the capacity to alter nuclear receptor expression (modulators) and/or serve as ligands for the receptors (agonists or antagonists), and thus can have synergistic or antagonistic effects on the expression of drug-metabolizing enzymes and transporters. Co-administration of drugs that are nuclear receptor agonists or antagonists can lead to severe toxicity, a loss of therapeutic efficacy or an imbalance in physiological substrates, providing a novel molecular mechanism for drug-drug interactions<sup>[20]</sup>.

### **RNA interference**

Authors suggested that the lack of p53 response may confer a growth advantage on preneoplastic hepatocytes and may be an important factor in hepatic tumor promotion by 2-acetylaminofluorene and other genotoxic compounds. Inhibition of RNA polymerase II driven transcription by DNA lesions may constitute one of the mechanisms leading to accumulation of the tumor suppressor p53<sup>[21]</sup>.

### **Oxidative stress and lipid peroxidation**

Redox signals are important in the modulation of cell function. Reactive oxygen species (ROS) generation influences many signaling proteins, interfering with molecular and biochemical processes responsible for cell differentiation, proliferation and death. Protein kinase (PKC) is a crucial signaling protein which is subject to redox regulation and controls these responses. ROS are products of normal cellular metabolism and are recognized to be harmful or beneficial to living systems. The harmful effect of ROS is termed oxidative stress and it occurs when there is an overproduction of these species or a deficiency of antioxidants. Enzymatic systems that contribute to ROS formation in the liver include CYP monooxygenases and NADPH oxidase. In the healthy liver, hepatocytes produce low amounts of ROS and Kupffer cells are well equipped to release ROS in response to infection. Antioxidants such as superoxide dismutase and catalase efficiently remove ROS surplus to maintain the normal cell homeostasis. Different stimuli are able to modify the redox state of liver cells modulating signal transduction pathways able to trigger many aspects of liver pathologies. In a recent study, evidence of the central role of PKC as a redox-sensitive molecule implicated in the pathogenesis and progression of toxic liver diseases was provided<sup>[22]</sup>.

The free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in membranes, setting off a free radical chain reaction sequence. Lipid peroxidation is known to cause membrane disruption, resulting in the loss of membrane integrity and leakage of microsomal enzymes. By-products of lipid peroxidation include reactive aldehydes that can form protein and DNA adducts and may contribute to hepatotoxicity and carcinogenicity, respectively. Natural antioxidants, including glutathione, are capable of quenching the lipid peroxidation reaction<sup>[23]</sup>.

### **Immune-mediated hypersensitivity**

Because liver undergoes continuous exposure to xenobiot-

ics, it possesses a variety of local immune mechanisms to face these challenges. In the liver there are both innate and adaptive immune cells, including tissue macrophages (KC), natural Killer (NK) cells, and non-NK (NKT) cells, which account for nearly 50% of intrahepatic leukocytes<sup>[24]</sup>. KC produce various cytokines and other mediators, including prostanooids, nitric oxide and ROS that play roles in promoting and regulating hepatic inflammation. Furthermore, KC represent a major population of antigen-presenting cells (APCs) having an important role in the balance between the induction of immunity and tolerance within the liver. It has been demonstrated that freshly isolated liver NK cells spontaneously induce the cytotoxicity of various cell lines, whereas NKT cells are cytotoxic in the presence of interleukin (IL)-2<sup>[25]</sup>. This cytotoxicity is further enhanced by IL-12 and IL-18, which are produced by activated KC. Another function ascribed to NK and NKT cells is their ability to produce high levels of T helper (Th) 1 and Th2 cytokines upon stimulation<sup>[26]</sup>. NK cells have been shown to represent a major source of interferon (IFN)- $\gamma$  in many types of liver disease<sup>[27]</sup>. NKT cells produce either IFN- $\gamma$  or IL-4, or in some cases both cytokines, depending on the differentiation state of the cells and the stimuli<sup>[25]</sup>. It has also been demonstrated that IL-4 produced by NKT cells may be associated with the initiation and regulation of Th2 responses. Various mechanisms have been suggested to explain this tolerance<sup>[28]</sup>, such as immune deviation, active suppression and apoptosis of activated T cells. Regarding immune deviation, it has been shown that Th2 cytokine production is preferentially maintained when adoptively transferred Th1 and Th2 cells are recovered from the liver. It has also been reported that liver sinusoidal endothelial cells (LSEC) are capable of selectively suppressing IFN- $\gamma$ -producing Th1 cells while concurrently promoting the outgrowth of IL-4-expressing Th2 cells<sup>[29]</sup>. Good evidence suggests that hepatic dendritic cells are also important in the induction of tolerance, rather than the activation of T-cell responses. It has been further demonstrated that although LSEC are capable of presenting antigens to T cells, LSEC-activated CD4<sup>+</sup> or CD8<sup>+</sup> T cells fail to differentiate into Th1 cells or cytotoxic effector cells, respectively<sup>[30]</sup>. Most drugs are not chemically reactive but can be activated metabolically to reactive species which, after binding to cellular macromolecules, become immunogenic and can elicit an effective immune response. Immune-mediated mechanisms have been proposed for idiosyncratic reactions observed with sulfonamides, halothane and phenytoins. Presentation of drug-protein adducts by professional cells to Th lymphocytes, and/or a direct association between the drug and major histocompatibility complex (MHC) proteins of hepatocytes could be involved in the activation of the immune system. As a consequence of this, drug-directed antibodies and/or T-lymphocytes able to recognize drug-derived haptens arise which are responsible for the clinical manifestations of hepatitis. Drug-directed antibodies can be detected in sera of allergic patients by solid-phase immunoassays. Sensitized T-lymphocytes can be shown by hapten-induced cell proliferation experiments and by the early expression of CD69 antigen<sup>[31]</sup>. Despite the



possible detection of drug-specific antibodies, it is difficult to directly prove the pathogenic role of the adaptive immune system in DILI, partly because of the lack of animal models. A difficulty in developing animal models is the fact that the default response of the liver to antigens is immunological tolerance. This could also explain the relatively low occurrence of this type of DILI in human beings.

### **Inflammation**

A recent paper emphasizes the imbalance between Th1 cells producing cytokines associated with a cell-mediated response and Th2 cells associated with an antibody response, leading to a shift in immune response to one that may participate in DILI during administration of certain drugs, especially in subjects with genetic polymorphisms in drug-metabolizing enzymes. In fact, several cases of DILI related to administration of drugs appear to be initiated or intensified by respiratory inflammation states, which stimulate sometimes dysregulated production of IFN- $\gamma$  and/or other proinflammatory cytokines/growth factors. This ends up in down-regulation of various induced and constitutive isoforms of CYPs and other enzymes involved in the metabolism of several drugs, thus having an important impact on the alterations in bioactivation and detoxication processes. DILI may eventually be prevented by screening methods that can identify genetic polymorphisms of drug-metabolizing enzymes and gene polymorphisms or RNA-expression profiles of some pro-inflammatory cytokines before patients take any drug<sup>[32]</sup>.

### **Apoptosis**

Although CYP-generated reactive metabolites can cause hepatocyte apoptosis, the mechanism of this effect has only recently been elucidated. Male rat hepatocytes were incubated with skullcap diterpenoids. This treatment decreased cellular glutathione and protein thiols and increased cellular  $\text{Ca}^{2+}$ . This activated  $\text{Ca}^{2+}$ -dependent tissue transglutaminase formed a cross-linked protein scaffold, and also opened the mitochondrial permeability transition pore, causing outer mitochondrial membrane rupture, increased cytosolic cytochrome c, activation of procaspase 3, internucleosomal DNA fragmentation, and ultrastructural features of apoptosis. Cell death was increased by a CYP3A inducer (dexamethasone) increasing glutathione depletion. In contrast, cell death was prevented by decreasing CYP3A activity (with troleandomycin), preventing glutathione depletion (with cysteine), blocking  $\text{Ca}^{2+}$ -modulated events (with calmidazolium), preventing mitochondrial permeability transition (with cyclosporin A), or inhibiting caspase 3 (with acetyl-Asp-Glu-Va-Asp-a dehyde). Both calmidazolium and cyclosporin A also prevented the increase in cytosolic cytochrome c and procaspase 3 activation<sup>[33]</sup>.

### **Calcium homeostasis imbalance**

When glutathione and other antioxidants are depleted, however, opportunities for lipid peroxidation are enhanced. Weakened cellular membranes allow sufficient leakage of  $\text{Ca}^{2+}$  into the cytosol to disrupt intracellular

$\text{Ca}^{2+}$  homeostasis. High  $\text{Ca}^{2+}$  levels in the cytosol activate  $\text{Ca}^{2+}$ -dependent proteases and phospholipases that further increase the breakdown of the membranes. Similarly, the increase in intracellular  $\text{Ca}^{2+}$  can activate endonucleases that can cause chromosomal damage and also contribute to cell death. Sustained cell regeneration and proliferation following cell death may increase the likelihood of unrepaired spontaneous, lipid peroxidation- or endonuclease-derived mutations that can lead to cancer.

## **FACTORS INFLUENCING PATIENT SUSCEPTIBILITY TO HEPATOXICITY**

Determination of DILI also includes an individual susceptibility. This susceptibility is governed by genetic, pre-existing and environmental factors. Predisposing factors are generally thought to be important to somehow explain the unpredictability of the phenomena through which substances turn into hepatotoxins, and consist of ethnic and racial factors, CYP polymorphisms, concomitant liver diseases, age, nutritional status and diet, gender and pregnancy.

### **Ethnic and racial factors**

These factors have important implications for susceptibility to acetaminophen hepatotoxicity following overdose especially in a small subgroup showing extensive metabolic activation. An exemplary study indicates markedly reduced metabolic activation of acetaminophen in Africans. These ethnic differences in acetaminophen metabolism may be related to genetics even though environmental factors, including differences in diet and protein intake, should not be excluded. There were no ethnic differences in the sulphate conjugation of acetaminophen, but the mean fractional recovery of the glucuronide conjugate in Caucasians was less than in Africans<sup>[34]</sup>.

### **Concomitant chronic liver diseases**

In liver diseases, pharmacokinetics are generally impaired. Pathogenetic factors include alterations in intestinal absorption, plasma protein binding, hepatic extraction ratio, liver blood flow and portal-systemic shunting, biliary excretion, enterohepatic circulation, and renal clearance. The key point is, however, the reduction of functional hepatic mass that may have complex effects on drug clearance, particularly biotransformation. Net results for an individual drug are unpredictable and do not correlate well with the type of liver damage, its severity, or liver laboratory test results. Thus, no general rules are available for modifying drug dosage in patients with liver disease. Recently, NonAlcoholic Fatty Liver Disease (NAFLD) has been found to be a fertile soil for the development of hepatotoxicity. With NAFLD now linked to obesity and metabolic syndrome, the impact of this observation should not be overlooked<sup>[35]</sup>.

Oxidative stress has been detected in patients affected by alcohol abuse, hepatitis C virus (HCV) infections, iron overload and chronic cholestasis<sup>[36]</sup>. Alcohol-induced liver

disease (ALD) has been associated with the synergistic induction of oxidative stress by alcohol metabolites, iron accumulation and antioxidant depletion<sup>[37]</sup>.

HCV infection may generate oxidative stress by chronic inflammation and by disruption of glutathione efflux. Therefore, oxidative stress is not only a consequence of chronic liver injury but it also contributes to fibrogenesis and it appears as a key player in the pathogenesis of hepatic diseases. Vitamin E could prevent the decrease in O<sub>2</sub> uptake<sup>[38]</sup>.

But what is the role of stress in determining hepatotoxicity? Its regulation of several enzymatic systems which are involved in the biotransformation of xenobiotics in the liver was recently investigated in a study using restraint stress as a stress model in animals. The results demonstrated that stress suppressed total basal CYP content of one third of animals and basal ethoxyresorufin 7-dealkylase activity. On the other hand, restraint stress increased total CYP content in 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene-treated mice, while slightly suppressing PROD activity. In addition, CYP2E1 dependent p-nitrophenol hydroxylation was suppressed by stress in the same animals and cytosolic aldehyde dehydrogenases were not affected. Although stress had no effect on basal CYP2A5 activity, the inducibility of this hepatic activity increased 2-fold after stress exposure. In addition, a slight suppression in liver glutathione content was found. Northern blot analysis revealed that restraint stress had a relatively suppressive effect on control CYP1A2 expression in the liver. In conclusion, stress was found to significantly interfere with the expression processes of some CYP450s<sup>[39]</sup>.

### Age

Increased risk of DILI may result from age-related changes in pharmacokinetics or pharmacodynamics. Overall hepatic metabolism of many drugs through the CYP enzyme system decreases with aging. For drugs with decreased hepatic metabolism, clearance typically decreases by 30%-40%. Theoretically, maintenance drug doses should be decreased by this percentage; however, the rate of drug metabolism varies greatly from person to person, and individual titration is required. Risk of any adverse effect and obviously of DILI increases exponentially with the number of drugs used, partly because multiple drug therapy reflects the presence of many diseases and increases risk of drug-disease and drug-drug interactions.

### Nutritional status and diet

Increasing evidence implicates dietary factors in the progression of diseases, including certain cancers, diabetes and obesity. Diet also regulates the expression and function of CYP genes which impacts on drug elimination and may also significantly affect disease pathogenesis. Upregulation of CYPs 2E1 and 3A4 occurs after feeding of experimental diets that are high in fats or carbohydrates; these diets also promote hepatic lipid infiltration, which is a component of the metabolic syndrome that characterizes obesity. Increased availability of lipid substrates for CYPs can enhance free radical production

and exacerbate tissue injury. Similar processes may also occur in other models of experimental disease states that exhibit a component of altered nutrient utilization. Food-derived chemicals, including constituents of cruciferous vegetables and fruits, modulate CYP expression and the expression of genes that encode cytoprotective phase II enzymes. Certain dietary indoles and flavonoids activate CYP1A expression either by direct ligand interaction with the aryl hydrocarbon receptor (AhR) or by augmenting the interaction of the AhR with xenobiotic response elements in CYP1A1 and other target genes. Other dietary chemicals, including methylenedioxypheyl (MDP) compounds and isothiocyanates also modulate CYP gene expression. Apart from altered CYP regulation, a number of dietary agents also inhibit CYP enzyme activity, leading to pharmacokinetic interactions with coadministered drugs. A well described example is that of grapefruit juice, which contains psoralens and possibly other chemicals that inactivate intestinal CYP3A4. Decreased presystemic oxidation by this CYP increases the systemic bioavailability of drug substrates and the likelihood of drug toxicity. Dietary interactions may complicate drug therapy but inhibition of certain CYP reactions may also protect the individual against toxic metabolites and free radicals generated by CYPs. Chemicals in teas and cruciferous vegetables may also inhibit human CYP enzymes that have been implicated in the bioactivation of chemical carcinogens. Thus, food constituents modulate CYP expression and function by a range of mechanisms, with the potential for both deleterious and beneficial outcomes<sup>[40]</sup>.

As previously emphasised, obesity is considered an important risk factor for DILI. Paradoxically, earlier studies have shown highly exaggerated mechanism-based liver injury by thioacetamide (TA) in rats following moderate diet restriction (DR). The objective of this significant study was to investigate the mechanism of higher liver injury of TA in DR rats. When male rats were maintained on DR (35% of ad libitum diet for 21 d), the total hepatic CYP was increased 2-fold along and there was a 4.6-fold increase in CYP2E1 protein, which corresponded with a 3-fold increase in CYP2E1 activity as measured by chlorzoxazone hydroxylation. To further test the involvement of CYP2E1, 24 and 18 h after pretreatment with pyridine (PYR) and isoniazid (INZ), specific inducers of CYP2E1, rats received a single administration of 50 mg of TA/kg. TA liver injury was > 2.5- and > 3-fold higher at 24 h in PYR + TA and INZ + TA groups, respectively, compared with the rats receiving TA alone. Pyridine pretreatment resulted in significantly increased total CYP content accompanied by a 2.2-fold increase in CYP2E1 protein and 2-fold increase in enzyme activity concordant with increased liver injury of TA, suggesting mechanism-based bioactivation of TA by CYP2E1. Hepatic injury of TA in DR rats pretreated with diallyl sulfide (DAS), a well known irreversible *in vivo* inhibitor of CYP2E1, was significantly decreased (60%) at 24 h. Carbon tetrachloride (CCl<sub>4</sub>), a known substrate of CYP2E1, caused less liver injury and greater animal survival, confirming inhibition of CYP2E1 by DAS pretreatment<sup>[41]</sup>.

### Gender

Some authors clarified this issue, studying 50 patients diagnosed with active tuberculosis infection with normal pretreatment liver laboratory tests; they were monitored clinically as well as biochemically in a prospective cohort analysis. Antitubercular drug-induced hepatotoxicity was found more often in females (OR 4.2). Younger patients were also at a higher risk (OR 2.75). Nutritional status, assessed by body mass index and serum albumin level, was the next most common predisposing factor<sup>[42]</sup>.

## PHENOTYPING AND GENOTYPING STUDIES

The genetic polymorphisms of human drug metabolizing enzymes have been firmly established. Based on the metabolic handling of certain probe drugs, the population can be divided into two phenotypes: the rapid acetylator/extensive metabolizer (EM) and slow acetylator/poor metabolizer (PM). For some authors, a quadri-modal behaviour is possible, that is, poor, intermediate, extensive and ultrarapid.

These polymorphisms have provided useful tools to study the relationship between genetically determined differences in the activity of drug metabolizing enzymes and the risk for adverse drug reactions and certain types of chemically-induced diseases.

With regard to the susceptibility of the two phenotypes, DILI can be anticipated for the following scenarios: (1) the drug toxicity is caused by the parent compound and the elimination of the drug proceeds exclusively *via* the polymorphic enzyme, there being no alternate pathways of biotransformation available. Thus the poor metabolizer phenotype will be more prone to such a type of toxicity since, at the same level of exposure, this phenotype will accumulate the drug as a result of impaired metabolism; (2) the polymorphic pathway is a major route of detoxification, so impairment of this pathway shifts the metabolism to an alternate pathway *via* which a reactive intermediate is formed. In such a situation the PM phenotype constitutes a major risk factor for toxicity (i.e. INZ hepatotoxicity); the toxicity is mediated by a reactive intermediate generated by a polymorphic enzyme. Hence EMs are at a much higher risk than PMs of developing toxicity or cancer (for example, smokers)<sup>[43]</sup>.

A further problem with PMs that the same dose of drug could yield a sustained plasma concentration. Poor metabolism is especially problematic with drugs that have a narrow therapeutic index like debrisoquine, phenformin or captopril. It has been estimated that psychiatric patients with CYP2D6 deficiency encounter more adverse drug incidents than those who are EMs<sup>[44]</sup>.

With estimates of the percentage of pharmaceuticals that are subject to metabolism by the CYP in excess of 80%, the relative activities of these enzymes in various subpopulations and even in individual patients can have important ramifications in matters ranging from dose selection to prediction of toxicity to suitability of a new

chemical entity for continued drug development.

As previously emphasized, CYP1A2, 2C9, 2C19, 2D6 and 3A are the major isoforms responsible for the metabolism of more than 90% of marketed drugs. Polymorphism of drug metabolism represents an important source of interindividual and interethnic variation in drug response.

CYP2D6, CYP2C9 and CYP2C19 are three polymorphic CYP enzymes. The EM phenotype occurs when there is at least one wild type allele at the relevant gene locus. The PM phenotype occurs when both alleles of either CYP2D6 or CYP2C19 carry inactivating mutations and give rise to synthesis of enzyme with impaired activity or no synthesis of enzyme at all.

Phenotyping is often based on high-performance liquid chromatography (HPLC) such as the determination of the dextromethorphan/total dextrorphan molar ratios as metabolic ratios (MRs) in plasma samples collected at 3 h after oral administration of 30 mg dextromethorphan hydrobromide. In this situation PMs and extensive EMs can be identified distinctly. To determine the real-time activity of the CYP isozymes, specific probe drugs can be employed. Recently, the use of multiple probe drugs, that is, a 'cocktail' approach, has become popular in pharmacogenetic studies as this provides a high-throughput approach in evaluating CYP isozyme activities. A number of cocktails (from five to six drugs) have been described in the literature.

These include the Pittsburgh cocktail, GW cocktail, Cooperstown cocktail and Karolinska cocktail. So far most of the analytical methods for these cocktails usually require a separate HPLC, gas chromatography (GC) or liquid chromatography/mass spectrometry (LC/MS) technique for each probe drug and its metabolite.

Recently, a fast gradient LC/MS method for the simultaneous determination of CYP substrates and metabolites in the GW cocktail was reported<sup>[45]</sup>. However, this cocktail has several practical limitations. First of all, the use of diclofenac as a CYP2C9 marker is undesirable due to its variable absorption in humans. Secondly, the use of mephenytoin is inconvenient as this drug is no longer commercially available in many parts of the world; besides, its sedative side effect is prominent, especially in PMs. Thirdly, chlorzoxazone, a probe drug in the cocktail, can significantly inhibit the CYP3A-mediated first-pass metabolism of midazolam in the gut and its use for the present purpose is not recommended.

A rapid LC/tandem MS method has been developed for the determination of six CYP probe substrate metabolites including acetaminophen for CYP1A2, 4-hydroxytolbutamide for CYP2C9, 5-hydroxyomeprazole for CYP2C19, dextrorphan for CYP2D6, 6-hydroxy-chlorzoxazone for CYP2E1 and dehydronifedipine for CYP3A4<sup>[46]</sup>.

What is the clinical importance of studying the CYP polymorphisms? It has been shown that the cholesterol-lowering effect as well as the efficacy and tolerability of simvastatin is influenced by CYP2D6 genetic polymorphism<sup>[47]</sup>. Because the different HMG-CoA reductase in-

hibitors differ with respect to the degree of metabolism by the different CYP enzymes, genotyping may help to select the appropriate HMG-CoA reductase inhibitor and the optimal dosage during the start of the treatment and will allow for more efficient individual therapy, also taking in account the eventual DILI. To clarify this point, CYP polymorphisms of fluvastatin were studied.

More than the hepatotoxicity, the pharmacokinetics of both enantiomers of fluvastatin depended on the CYP2C9 genotype with a 3-fold mean difference in the active enantiomer and even greater differences in the inactive enantiomer. Differences in plasma concentrations were not reflected in cholesterol lowering after 14 d of fluvastatin intake in healthy volunteers<sup>[48]</sup>. In fact, the authors did not find any evidence to support CYP2C9 and CYP2C19 genetic polymorphisms as predictable potential risk factors for DILI<sup>[49]</sup>.

## PREDICTING VARIABILITY IN PHARMACOKINETICS

Obviously, it is impossible to genotype all individuals due to the cost, even though for some drugs it should be taken into serious consideration because a single serious incident (hepatic failure) could lead to an excessive health care involvement (liver transplantation). Several examples have been reported.

CYP2C19 is highly polymorphic, with variations in both the expression of mRNA and enzyme, plus actual differences in the protein coding region that give rise to differing rates of catalysis. As with most polymorphisms, there appear to be differences in expression in different ethnic groups. For example, the frequency of PMs among Asians is nearly 20% of Caucasians<sup>[50]</sup>. The proton pump inhibitor omeprazole and related ulcer drugs are oxidized by CYP2C19, and PMs show a better response to these drugs<sup>[51]</sup>.

Not all drug interactions are genetically determined. In some cases, an inhibitor can block metabolism of a drug and produce the same effect as would poor metabolism. In retrospect, every marketed drug is a relatively successful drug in terms of the limited problems associated with widespread use, the deaths of some individuals notwithstanding-as in the case of “ultra” rapid metabolizers-or, more commonly, arising from enzyme induction. A classic example involves CYP3A4 and 17 $\alpha$ -ethynylestradiol, the estrogenic component of oral contraceptives. Similarly, hyperforin, a potent CYP inducer found in the herbal medicine St. John’s wort, greatly increases the expression of CYPs that metabolize drugs used for AIDS treatment and organ transplantation. Cases for enhanced drug toxicity due to elevated levels of CYPs are probably less clear; however, CYP3A4 converts the antidiabetic drug troglitazone into toxic products, although the mechanism of toxicity is still unclear. Troglitazone has since been removed from the market. In any event, the drug development process now incorporates a variety of *in vitro* studies designed to predict bioavailability, inhibition of CYP reaction, and the effects

of any induction prior to consideration of clinical trials. Conclusively, genotyping can provide useful information about the expected behavior of a drug.

## HAS CYP A ROLE IN THE “DIRECT” HEPATOTOXICITY?

CCl<sub>4</sub> is a well-known model compound for producing chemical hepatic injury. CYP is an important monooxygenase in biology. Recent research investigated CYP protein expression in the *in vivo* hepatotoxicity of rats induced by CCl<sub>4</sub>. In this experiment, CCl<sub>4</sub> was administered to male rats, and their livers at 24 h post-dosing were studied using proteomic analysis. Blood biochemistry and histopathology were examined to identify specific changes. At the same time, a novel acetylation stable isotopic labeling method coupled with LTQ-FTICR MS was applied to disclose the changes in CYP expression amounts. The quantitative proteomics method demonstrated its correlation coefficient was 0.9998 in a 100-fold dynamic range and the average ratio of the labeled peptides was 1.04, which was very close to the theoretical ratio of 1.00 and the standard deviation (SD) of 0.21. With this approach, 17 CYP proteins were identified and quantified with high confidence. Among them, the expression amounts of 2C11, 3A2, and 2 E1 were down-regulated, while those of 2C6, 2B2, and 2B1 were up-regulated<sup>[52]</sup>.

## AN EXAMPLE OF DAMAGE MECHANISMS

CCl<sub>4</sub> continues to provide an important service today as a model substance to elucidate the mechanisms of action of hepatotoxic effects such as fatty degeneration, fibrosis, hepatocellular death, and carcinogenicity. CCl<sub>4</sub> is activated by CYP2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form the trichloromethyl radical, CCl<sub>3</sub>\*. This radical can bind to cellular molecules (protein, lipid, nucleic acids), impairing crucial cellular processes such as lipid metabolism, leading to fatty degeneration (hepatic steatosis). Adduct formation between CCl<sub>3</sub>\* and DNA is thought to possibly induce hepatic cancer. This radical can also react with O<sub>2</sub> to form the trichloromethylperoxy radical CCl<sub>3</sub>OO\*, a highly reactive species, also called ROS. CCl<sub>3</sub>OO\* initiates the chain reaction of lipid peroxidation, which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular Ca<sup>2+</sup> sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Among the degradation products of fatty acids are reactive aldehydes, especially 4-hydroxynonenal, which bind easily to functional groups of proteins and inhibit important enzyme activities. CCl<sub>4</sub> intoxication also leads to hypomethylation of cellular components; in the case of RNA the outcome is thought to be inhibition of protein synthesis, and in the



case of phospholipids it plays a role in the inhibition of lipoprotein secretion. None of these processes per se is considered the ultimate cause of CCl<sub>4</sub>-induced cell death; it is by cooperation that they achieve a fatal outcome, provided the toxicant acts in a high single dose, or over longer periods of time at low doses. At the molecular level CCl<sub>4</sub> activates tumor necrosis factor (TNF)- $\alpha$ , nitric oxide, and transforming growth factor (TGF)- $\alpha$  and - $\beta$  in the cell, processes that appear to direct the cell primarily toward (self-)destruction or fibrosis. TNF- $\alpha$  pushes toward apoptosis, whereas TGF- $\beta$  appears to direct toward fibrosis. IL-6, although induced by TNF- $\alpha$ , has a clearly antiapoptotic effect, and IL-10 also counteracts TNF- $\alpha$  action. Thus, both interleukins have the potential to initiate recovery of the CCl<sub>4</sub>-damaged hepatocyte. Several of the above-mentioned toxicological processes can be specifically interrupted with the use of antioxidants and mitogens, respectively, by restoring cellular methylation, or by preserving calcium sequestration. Chemicals that induce CYPs that metabolize CCl<sub>4</sub>, or delay tissue regeneration when co-administered with CCl<sub>4</sub> will potentiate its toxicity thoroughly, while appropriate CYP inhibitors will alleviate much of the toxicity. O<sub>2</sub> partial pressure can also direct the course of CCl<sub>4</sub> hepatotoxicity. Pressures between 5 and 35 mmHg favor lipid peroxidation, whereas the absence of O<sub>2</sub>, as well as a partial pressure above 100 mmHg, both prevent lipid peroxidation entirely. Consequently, the location of CCl<sub>4</sub>-induced damage mirrors the O<sub>2</sub> gradient across the liver lobule. Mixed halogenated methanes and ethanes, found as so-called disinfectant by-products at low concentrations in drinking water, elicit symptoms of toxicity very similar to CCl<sub>4</sub>, including carcinogenicity<sup>[23]</sup>.

## TOTAL DOSE OF DRUG

Among drugs that are feared of inducing hepatotoxicity, mainly when taken for a very long period, statins are largely under-dosed, but they do in rare cases cause significant liver injury whereas antiretroviral therapy is associated with hepatotoxicity in 10% of treated patients<sup>[16]</sup>.

In a previous study, liver morphology was examined in 41 patients with vitamin A hepatotoxicity. Cirrhosis was found in 17, mild chronic hepatitis in 10, noncirrhotic portal hypertension in 5, and "increased storage" alone in nine cases. During a mean follow-up period of 4.6 years, six patients died of causes related to the liver disease. A precise appraisal of drug consumption was obtained in 29 cases. Among them the total cumulative intake was highest in patients with cirrhosis ( $423 \pm 103 \times 10^6$  IU) and significantly lower in those with noncirrhotic liver disease ( $88.5 \pm 41$ ;  $P < 0.02$ ). The smallest continuous daily consumption leading to cirrhosis was 25000 IU during 6 years, whereas higher daily doses ( $\geq 100000$  IU) taken over 2.5 years resulted in similar histological lesions. It was concluded that prolonged and continuous consumption of doses in the low "therapeutic" range can result in life-threatening liver damage<sup>[53]</sup>.

Recently, an interesting paper, reporting data from both USA and Sweden, showed a clear relationship be-

tween daily doses of oral prescription medications and idiosyncratic DILI, particularly as regards daily doses  $> 50$  mg/d<sup>[54]</sup>.

## INDUCTION AND INHIBITION OF CYP ACTIVITY

The possible pharmacokinetic consequences of enzyme induction depend on the localization of the enzyme. They include decreased or absent bioavailability for orally administered drugs, increased hepatic clearance or accelerated formation of reactive metabolites, which is usually related to local toxicity. The toxicological consequences of enzyme induction in humans are fortunately rare, and appear to be mainly limited to hepatotoxicity in ethanol-type induction<sup>[55]</sup>.

Diclofenac sodium (DF-Na) is an NSAID used in various aspects of inflammatory disease. The effects of phenobarbital (PB) on metabolism and toxicity of DF-Na *in vitro* and the potential mechanism of DF-Na induced hepatotoxicity have been examined. The decline of CYP 3A was partially reversed by CYP inducer PB, and the maximum induction of CYP 3A was 2.2-fold over control after continuous exposure of hepatocytes to 2 mmol/L PB for 48 h. These findings suggest that the hepatotoxicity and metabolism of DF-Na in rat hepatocytes are increased when hepatic CYP 3A activity is increased<sup>[56]</sup>. Itraconazole and fluconazole, two antifungal drugs with potent inhibitory effect on CYP, induce hepatotoxicity clinically, but the mechanism underlying the hepatotoxicity is unknown. Pretreatment with SKF 525A, an inhibitor of CYPs, induced more severe hepatotoxicity with both itraconazole and fluconazole *in vivo*<sup>[57]</sup>.

## THE IMPACT OF DILI ON DRUG DEVELOPMENT

The inability to predict if a metabolically bioactivated compound will cause toxicity in later stages of drug development or post-marketing is of great importance. One approach for improving the predictive success of compound toxicity could be to compare the gene expression profile in preclinical models dosed with novel compounds to a gene expression database generated from compounds with known toxicity.

A current study<sup>[58]</sup> in mice utilized a known hepatotoxic compound N-methylformamide and its two analogs labeled with deuterium at different positions to block metabolic oxidation at the formyl [d (1)] and methyl [d (3)] moieties. The data set generated from the different groups of animals enabled authors to determine which gene expression changes were attributed to the bioactivating pathway. The metabolic pathway leading to the production of reactive methyl isocyanate resulted in distinct expression patterns that correlated with histopathologic findings. There was a clear correlation between the expression of certain genes involved in the cell cycle/apoptosis and inflammatory pathways and

the presence of reactive metabolite. These genes may serve as potential genomic biomarkers of hepatotoxicity induced by soft-electrophile-producing compounds. However, the robustness of these potential genomic biomarkers will need to be validated before being adopted into the drug discovery screening process.

## AN OPINION ABOUT WIDELY USED DRUGS

Although liver injury has been associated with the “statins”, the frequency of such toxicity is lower than that of the control population and the value of biochemical monitoring remains unproved<sup>[59]</sup>. Clinicians may be concerned about prescribing statins to patients with chronic liver disease, but there is little evidence to suggest that DILI from statins is increased in these patients. Thus, we should prescribe statins for the same indications in patients with chronic liver disease as in patients without, but with closer monitoring<sup>[60]</sup>.

## HERBAL REMEDIES AND OTHER DIETARY SUPPLEMENTS

A dietary supplement is defined as any product in pill or liquid form containing a herb, vitamin, amino acid or mineral that complements the normal diet. Indeed, every agency regulates dietary supplements differently from drugs, i.e. only ensuring quality control and good manufacturing processes but not standardization of the active ingredients. Dietary supplements are commonly used primarily because they are widely available and can be bought without consulting a physician. However, a few supplements are now proven to be safe and useful complements to standard drugs. The supplement may have unlisted ingredients, which may be inert or harmful, or it may contain variable amounts of active ingredients, especially when whole herbs are ground or made into extracts. Most herbal products are mixtures of several substances, and which ingredients are active is not always known. Additional areas of concern include stability of supplements (especially herbal products) once manufactured, use of dietary supplements instead of conventional drugs, toxicity in children and the elderly, and interactions between supplements and drugs. Patients may not think to disclose or may wish to conceal their use of dietary supplements. For this reason, the patient's history should periodically include explicit questions about past and recent consumption of dietary supplements. Recently the use of herbal preparations as remedies for various medical conditions has been increasing rapidly. In one study, 38.9% of patients with chronic liver disease were found to use some sort of herbal preparation<sup>[61]</sup>.

Efficacy and safety of medicinal plants naturally represent the object of interest for pharmacologists, and it is surely this aspect which gives the most important information on herbal medicine use<sup>[62]</sup>.

Many plants have been studied and results published

showing variable degrees of efficacy. Toxicity aspects of some of the most frequently used plants are now well known<sup>[63]</sup>.

Among others, a recent report emphasizes the potentially severe hepatotoxicity of Kava which has recently led to the retraction of Kava-containing drugs by the pharmacovigilance authorities in Germany<sup>[64]</sup>. Authors reported two cases of acute liver injury along with the intake of greater celandine (*Chelidonium majus*), a well-known herbal remedy frequently used for irritable bowel syndrome. All other possible causes of acute liver damage were excluded in both patients. In one patient, cholestatic hepatitis recurred rapidly after involuntary re-exposure. Both patients fully recovered after the withdrawal of greater celandine. The two cases add to the existing database about the potential hepatotoxicity of drugs containing greater celandine and raise the question whether the approval of this drug should be re-evaluated in the light of a lack of evidence for a therapeutic benefit<sup>[65]</sup>.

## SOME CONSIDERATIONS ON CAUSALITY ASSESSMENT

Many methods have been proposed to assess the individual causality between a drug treatment and the occurrence of adverse drug events (ADRs), including hepatotoxicity. Briefly, these methods may be classified into the following approaches, i.e. expert judgement, probabilistic methods and algorithms.

In expert judgement or global introspection (GI), an expert expresses a judgement about possible drug causation after having taken into account all the available and relevant information on the considered case.

Theoretically, it is possible to apply pre-existing Causality Assessment Methods (CAMs) to the assessment of causality in cases with diagnostic difficulties.

We have historical scales such as Naranjo probability scale<sup>[66]</sup>, Danan's international consensus criteria<sup>[67]</sup>, Maria's and Victorino's scales<sup>[68]</sup> or Beers criteria for ADRs<sup>[69]</sup> to make such events predictable and often preventable. Still, on the basis of a global score, four categories of preventability of ADRs (“preventable”, “potentially preventable”, “unclassable”, “not preventable”) were proposed by other researchers<sup>[70]</sup>.

Standards are lacking for validation of drug CAMs. An original model has been proposed using a positive rechallenge as an external standard<sup>[71]</sup>. The GI approach suffers from marked subjectivity leading to poor reproducibility and intra- and inter-rater disagreements. A study confirms that experts express marked disagreements when assessing drug causality independently. The agreement rate was lower for intermediate levels of causality, especially when strong evidence was lacking for confirming or ruling out drug causality<sup>[72]</sup>. Probabilistic methods are usually regarded as the most rigorous<sup>[73]</sup>. The probabilistic approach is based on the Bayes theorem and makes it possible to directly assess the odds of drug causation. However, these methods are rather troublesome to routinely use because information for

assessing the probability of drug causation is rarely available. Unlike the Bayesian approach, algorithms have appealing simplicity and are much more widely used for the operational assessment of ADRs. The main reason for their use is to increase inter- and intra-rater agreement. The overall observed agreement between algorithm and GI was moderate although poorly different from chance, confounding variables being a shortcoming of algorithms ability in assessing causality<sup>[74]</sup>.

### Drawbacks of animal models

The doubtful assumption that animal models are reasonably predictive of human outcomes has provided the basis for their widespread use in toxicity testing and in biomedical research aimed at developing cures for human diseases. To investigate the validity of this assumption, comprehensive bibliographic databases were searched for published systematic reviews of the human clinical or toxicological utility of animal experiments. In 20 reviews in which clinical utility was examined, the authors concluded that animal models were either significantly useful in contributing to the development of clinical interventions, or were substantially consistent with clinical outcomes, in only two cases, one of which was contentious. These reviews failed to clearly demonstrate utility in predicting human toxicological outcomes; consequently, animal data may not generally be assumed to be substantially useful for these purposes. Possible causes include interspecies differences, the distortion of outcomes arising from experimental environments and protocols, and the poor methodological quality of many animal experiments. What is more, very few reviews existed in which the majority of animal experiments were of good methodological quality. The poor human clinical and toxicological utility of most animal models for which data exists, in conjunction with economic costs, justify a perplexity on animal models<sup>[75]</sup>.

In numerous cases, researchers are simply not aware of the limitations of the animal experiment as such. For example, many animal experiments are dramatically “under-powered”, that is, carried out with groups that are too small to allow conclusions to be drawn from the outcome. This stands in marked contrast to *in vitro* experiments where replicate experiments usually represent no major problem. Since *in vitro* models are generally more prone to artefacts due to the numerous variables, for example, of cell culture, the key requirement for their application is their validation and quality control. Sadly, many methods, even if published in the scientific literature, are little standardised and reproducible. Due to limitations in space, many scientific journals cannot publish detailed methodological descriptions. However, nowadays a supplementary central deposit of methods could easily be linked to the respective article<sup>[76]</sup>.

## ETHICAL AND LEGAL PROBLEMS ABOUT DRUG-INDUCED LIVER INJURY

Patients should be especially cautious about combining

multiple drugs, and tell their doctor about any drugs or other substances they are taking, including prescription and over-the-counter medications, recreational drugs, herbal remedies, and nutritional supplements. Health care professionals are encouraged to report all ADRs, mainly hepatotoxicity, and to pay much more attention in prescribing and administering drugs.

Pharmacovigilance is a key step in discovering DILI. But, it is also concerned with pharmacological, pathological, epidemiological and legal respects, other ADRs and interactions as well as problems relating to ineffectiveness, inappropriate use, dependence or poisoning. Physicians should always think of this ADR in the absence of other clear hepatic disease.

## LIVER DISEASES POTENTIALLY CAUSED BY DRUGS

### Acute hepatitis

**Dose-dependent:** Acetaminophen<sup>[77,78]</sup>, salicylates<sup>[79]</sup>, (high doses i.e. > 2 g/d).

**Dose-independent:** Acebutolol<sup>[80]</sup>, allopurinol<sup>[81]</sup>, carbamazepine<sup>[82]</sup>, cimetidine<sup>[83]</sup>, dantrolene<sup>[84]</sup>, diclofenac<sup>[79]</sup>, ethambutol<sup>[85]</sup>, ethionamide<sup>[86]</sup>, enflurane<sup>[87]</sup>, phenelzine<sup>[88]</sup>, phenindione<sup>[89]</sup>, phenobarbital<sup>[90]</sup>, phenytoin<sup>[91]</sup>, phenylbutazone<sup>[79]</sup>, halothane<sup>[92]</sup>, ibuprofen<sup>[79]</sup>, indomethacin<sup>[79]</sup>, isoniazid<sup>[85]</sup>, ketoconazole<sup>[93]</sup>, labetalol<sup>[94]</sup>, maprotiline<sup>[95]</sup>, metoprolol<sup>[96]</sup>, mianserin<sup>[97]</sup>, naproxen<sup>[79]</sup>, para-aminosalicylic acid<sup>[98]</sup>, piroxicam<sup>[79]</sup>, pyrazinamide<sup>[85]</sup>, quinidine<sup>[99]</sup>, penicillins<sup>[100]</sup>, ranitidine<sup>[101]</sup>, sulfonamides<sup>[102]</sup>, sulindac<sup>[79]</sup>, tricyclic antidepressants<sup>[103]</sup>, trimethoprim-sulfamethoxazole<sup>[104]</sup>, valproic acid<sup>[105]</sup>, verapamil<sup>[106]</sup>.

### Acute fatty liver

Adrenocortical steroids<sup>[107]</sup>, phenytoin<sup>[108]</sup>, salicylates<sup>[79]</sup>.

### Fatty liver

Amiodarone<sup>[109]</sup>, asparaginase<sup>[110]</sup>, ibuprofen<sup>[79]</sup>, indometacin<sup>[79]</sup>, ketoconazole<sup>[111]</sup>, methylodopa<sup>[112]</sup>, naproxen<sup>[79]</sup>, nifedipine<sup>[113]</sup>, acetaminophen<sup>[77]</sup>, perhexiline<sup>[114]</sup>, rifampicin<sup>[85]</sup>, sulindac<sup>[79]</sup>, tetracyclin<sup>[115]</sup>, valproic acid<sup>[116]</sup>, zidovudin<sup>[117]</sup>.

### Cholestatic syndrome

Amoxicillin/clavulanate<sup>[118]</sup>, azathioprine<sup>[119]</sup>, captopril<sup>[120]</sup>, carbamazepine<sup>[121]</sup>, carbimazole<sup>[122]</sup>, cephalosporins<sup>[123]</sup>, chlordiazepoxide<sup>[124]</sup>, chlorpropamide<sup>[125]</sup>, cloxacillin<sup>[126]</sup>, cyclosporine<sup>[127]</sup>, danazol<sup>[128]</sup>, disopyramide<sup>[129]</sup>, enalapril<sup>[130]</sup>, erythromycin<sup>[131]</sup>, flecainide, flucoxacin<sup>[132]</sup>, flurazepam<sup>[133]</sup>, flutamide<sup>[134]</sup>, gold<sup>[135]</sup>, griseofulvin<sup>[136]</sup>, glyburide<sup>[137]</sup>, imipramine<sup>[138]</sup>, haloperidol<sup>[139]</sup>, ketoconazole<sup>[140]</sup>, megestrol<sup>[141]</sup>, mercaptopurine<sup>[119]</sup>, methimazole<sup>[142]</sup>, methyltestosterone<sup>[143]</sup>, nifedipine<sup>[144]</sup>, nitrofurantoin<sup>[145]</sup>, norethandrolone<sup>[146]</sup>, nonsteroidal anti-inflammatory drugs<sup>[79]</sup>, oral contraceptives<sup>[147]</sup>, phenothiazines<sup>[148]</sup>, phenytoin<sup>[149]</sup>, penicillamine<sup>[150]</sup>, propoxyphene<sup>[151]</sup>, sulfonamides<sup>[152]</sup>, tamoxifen<sup>[153]</sup>, thiabendazole<sup>[154]</sup>, tolbutamide<sup>[155]</sup>, tricyclic antidepressants<sup>[156]</sup>, troleandomycin<sup>[157]</sup>, verapamil<sup>[158]</sup>.



**Liver granulomas**

Allopurinol<sup>[159]</sup>, aspirin<sup>[79]</sup>, carbamazepine<sup>[160]</sup>, chlorpromazine<sup>[161]</sup>, diltiazem<sup>[162]</sup>, gold<sup>[163]</sup>, hydralazine<sup>[164]</sup>, nitrofurantoin<sup>[165]</sup>, penicillin<sup>[166]</sup>, phenylbutazone<sup>[79]</sup>, phenytoin<sup>[167]</sup>, pyrazinamide<sup>[168]</sup>, quinidine<sup>[169]</sup>, sulfasalazine<sup>[170]</sup>.

**Chronic liver disease**

Acetaminophen (in chronic use or at high doses)<sup>[79]</sup>, dantrolene<sup>[171]</sup>, isoniazid<sup>[172]</sup>, methyldopa<sup>[173]</sup>, phenytoin<sup>[174]</sup>.

**Liver cirrhosis/fibrosis**

Methotrexate<sup>[175]</sup>, nitrofurantoin<sup>[176]</sup>, terbinafine<sup>[177]</sup>.

**Liver tumors**

Anabolic steroids<sup>[178]</sup>, danazol<sup>[179]</sup>, oral contraceptives<sup>[178]</sup>, testosterone<sup>[178]</sup>, thiorast<sup>[180]</sup>.

**Vascular reactions**

Anabolic steroids<sup>[181]</sup>, azathioprine<sup>[182]</sup>, cyclophosphamide/cyclophosphamide combination<sup>[183]</sup>, dacarbazine<sup>[184]</sup>, oral contraceptives<sup>[185]</sup>, thioquanine<sup>[186]</sup>, vincristine<sup>[187]</sup>.

**Fulminant hepatitis and hepatic failure**

Lamotrigine<sup>[188]</sup>, nimesulide<sup>[189]</sup>, carbamazepine and levetiracetam<sup>[190]</sup>, isoniazid<sup>[191]</sup>, clarithromycin<sup>[192]</sup>, ecstasy<sup>[193]</sup>.

## LIVER DISEASES EVENTUALLY CAUSED BY DIETARY SUPPLEMENT (MAINLY IN OBESE PATIENTS)

Germander (*Teucrium chamaedrys*) extracts, widely used in Europe in the last decades as a weight loss agent, cause DILI probably mediated by furano neoclerodane diterpenoids<sup>[194]</sup>. Chaparral (creosote bush, greasewood, or *Larrea tridentata*) is a desert shrub traditionally used by Native Americans for treatment of several ailments. More recently, preparations of chaparral leaves have been marketed for use as weight loss agents. Reports of chaparral hepatotoxicity were first seen in 1992. The mechanism of chaparral toxicity is unclear but may involve its active ingredient, nordihydroguaiaretic acid<sup>[195]</sup>. Kava (kava kava, awa, or kew), derived from the dried root and rhizome of *Piper methysticum*, has recently been marketed as an anxiolytic and mood enhancer. Recent series from Europe have described more than 30 cases of kava-associated hepatic injury, including five cases leading to OLT. The mechanism of hepatic injury appears to be immune-mediated, with CYP2D6 deficiency perhaps being a risk factor<sup>[196]</sup>. *Ma huang* (from *Ephedra sinica* and other *Ephedra* species) is a traditional Chinese extract used for treatment of asthma, nasal congestion, and fever. Recent Western marketing has focused on the stimulatory effects of *Ma huang*, which contains 0.15%-2% of ephedrine-like alkaloids by weight. Although most adverse effects of *Ma huang* are cardiovascular or neurological (e.g. hypertension, stroke, myocardial infarction, seizures, and psychosis), 4% of reports mentioned acute

hepatitis. *Ma huang* contains phytochemicals which are thought to modify its toxic activity<sup>[197]</sup>. In addition to the above supplements, liver injury has been attributed to other botanical agents. The pyrrolizidine alkaloids found in comfrey leaves and *Heliotropium*, *Senecio*, and *Crotalaria* species are known to cause veno-occlusive disease of the liver *via* a toxic effect<sup>[198]</sup>. Mixtures of valerian and skullcap (*Valeriana officinalis* and *Scutellaria lateriflora*) have induced hepatitis *via* alkylating agents. LipoKinetix, was marketed as a dietary supplement for weight loss. Following reports of seven cases of severe hepatotoxicity associated with its use, the FDA moved to remove it from the market in November 2001. Hepatic injury appears to be due to an idiosyncratic reaction, perhaps related to phenylpropanolamine<sup>[199]</sup>. Among other weight loss agents, usnic acid should be suspected in cases of severe hepatotoxicity<sup>[200]</sup>.

For further details about the topics of this report, we advise the readers to consider the review by Bleibel *et al*<sup>[201]</sup> and/or to connect to: <http://www.fda.gov/cder/guidance/7507dft.htm>.

**REFERENCES**

- 1 Gunawan B, Kaplowitz N. Clinical perspectives on xenobiotic-induced hepatotoxicity. *Drug Metab Rev* 2004; **36**: 301-312
- 2 Liu YT, Hao HP, Liu CX, Wang GJ, Xie HG. Drugs as CYP3A probes, inducers, and inhibitors. *Drug Metab Rev* 2007; **39**: 699-721
- 3 Floby E, Briem S, Terelius Y, Sohlenius-Sternbeck AK. Use of a cocktail of probe substrates for drug-metabolizing enzymes for the assessment of the metabolic capacity of hepatocyte preparations. *Xenobiotica* 2004; **34**: 949-959
- 4 Dixit V, Hariparsad N, Desai P, Unadkat JD. In vitro LC-MS cocktail assays to simultaneously determine human cytochrome P450 activities. *Biopharm Drug Dispos* 2007; **28**: 257-262
- 5 Jones JO, Diamond MI. Design and implementation of cell-based assays to model human disease. *ACS Chem Biol* 2007; **2**: 718-724
- 6 Li J, Liu Y, Zhang JW, Wei H, Yang L. Characterization of hepatic drug-metabolizing activities of Bama miniature pigs (*Sus scrofa domestica*): comparison with human enzyme analogs. *Comp Med* 2006; **56**: 286-290
- 7 Fujita K, Kamataki T. Genetically engineered bacterial cells co-expressing human cytochrome P450 with NADPH-cytochrome P450 reductase: prediction of metabolism and toxicity of drugs in humans. *Drug Metab Pharmacokinet* 2002; **17**: 1-22
- 8 Meier Y, Cavallaro M, Roos M, Pauli-Magnus C, Folkers G, Meier PJ, Fattinger K. Incidence of drug-induced liver injury in medical inpatients. *Eur J Clin Pharmacol* 2005; **61**: 135-143
- 9 Andrade RJ, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, Garcia-Munoz B, Gonzalez-Grande R, Pizarro A, Duran JA, Jimenez M, Rodrigo L, Romero-Gomez M, Navarro JM, Planas R, Costa J, Borrás A, Soler A, Salmeron J, Martin-Vivaldi R. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* 2005; **129**: 512-521
- 10 De Valle MB, Av Klinteberg V, Alem N, Olsson R, Bjornsson E. Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic. *Aliment Pharmacol Ther* 2006; **24**: 1187-1195
- 11 Marti L, Del Olmo JA, Tosca J, Ornia E, Garcia-Torres ML, Serra MA, Rodriguez F, Lluch P, Escudero A, Rodrigo JM. Clinical evaluation of drug-induced hepatitis. *Rev Esp En-*



- ferm Dig* 2005; **97**: 258-265
- 12 **Chan KA**, Truman A, Gurwitz JH, Hurley JS, Martinson B, Platt R, Everhart JE, Moseley RH, Terrault N, Ackerson L, Selby JV. A cohort study of the incidence of serious acute liver injury in diabetic patients treated with hypoglycemic agents. *Arch Intern Med* 2003; **163**: 728-734
  - 13 **Aithal PG**, Day CP. The natural history of histologically proved drug induced liver disease. *Gut* 1999; **44**: 731-735
  - 14 **Garcia Rodriguez LA**, Ruigomez A, Jick H. A review of epidemiologic research on drug-induced acute liver injury using the general practice research data base in the United Kingdom. *Pharmacotherapy* 1997; **17**: 721-728
  - 15 **Li B**, Wang Z, Fang JJ, Xu CY, Chen WX. Evaluation of prognostic markers in severe drug-induced liver disease. *World J Gastroenterol* 2007; **13**: 628-632
  - 16 **Hussaini SH**, Farrington EA. Idiosyncratic drug-induced liver injury: an overview. *Expert Opin Drug Saf* 2007; **6**: 673-684
  - 17 **Kaplowitz N**. Biochemical and cellular mechanisms of toxic liver injury. *Semin Liver Dis* 2002; **22**: 137-144
  - 18 **Masubuchi Y**, Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. *J Hepatol* 2005; **42**: 110-116
  - 19 **Kon K**, Ikejima K, Okumura K, Aoyama T, Arai K, Takei Y, Lemasters JJ, Sato N. Role of apoptosis in acetaminophen hepatotoxicity. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S49-S52
  - 20 **Wang H**, LeCluyse EL. Role of orphan nuclear receptors in the regulation of drug-metabolizing enzymes. *Clin Pharmacokinet* 2003; **42**: 1331-1357
  - 21 **van Gijssel HE**, Mullenders LH, van Oosterwijk MF, Meerman JH. Blockage of transcription as a trigger for p53 accumulation by 2-acetylaminofluorene DNA-adducts. *Life Sci* 2003; **73**: 1759-1771
  - 22 **Jeong DH**, Lee SJ, Lee JH, Bae IH, Jeong KS, Jang JJ, Lim IK, Kim MR, Lee MJ, Lee YS. Subcellular redistribution of protein kinase C isozymes is associated with rat liver cirrhotic changes induced by carbon tetrachloride or thioacetamide. *J Gastroenterol Hepatol* 2001; **16**: 34-40
  - 23 **Weber LW**, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2003; **33**: 105-136
  - 24 **Mehal WZ**, Azzaroli F, Crispe IN. Immunology of the healthy liver: old questions and new insights. *Gastroenterology* 2001; **120**: 250-260
  - 25 **Doherty DG**, Norris S, Madrigal-Estebas L, McEntee G, Traynor O, Hegarty JE, O'Farrelly C. The human liver contains multiple populations of NK cells, T cells, and CD3+CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J Immunol* 1999; **163**: 2314-2321
  - 26 **Chen H**, Paul WE. Cultured NK1.1+ CD4+ T cells produce large amounts of IL-4 and IFN-gamma upon activation by anti-CD3 or CD1. *J Immunol* 1997; **159**: 2240-2249
  - 27 **Li Z**, Diehl AM. Innate immunity in the liver. *Curr Opin Gastroenterol* 2003; **19**: 565-571
  - 28 **Calne RY**, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; **223**: 472-476
  - 29 **Klugewitz K**, Blumenthal-Barby F, Schrage A, Knolle PA, Hamann A, Crispe IN. Immunomodulatory effects of the liver: deletion of activated CD4+ effector cells and suppression of IFN-gamma-producing cells after intravenous protein immunization. *J Immunol* 2002; **169**: 2407-2413
  - 30 **Limmer A**, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000; **6**: 1348-1354
  - 31 **Castell JV**. Allergic hepatitis: a drug-mediated organ-specific immune reaction. *Clin Exp Allergy* 1998; **28** Suppl 4: 13-19
  - 32 **Prandota J**. Important role of proinflammatory cytokines/other endogenous substances in drug-induced hepatotoxicity: depression of drug metabolism during infections/inflammation states, and genetic polymorphisms of drug-metabolizing enzymes/cytokines may markedly contribute to this pathology. *Am J Ther* 2005; **12**: 254-261
  - 33 **Haouzi D**, Lekehal M, Moreau A, Moulis C, Feldmann G, Robin MA, Letteron P, Fau D, Pessayre D. Cytochrome P450-generated reactive metabolites cause mitochondrial permeability transition, caspase activation, and apoptosis in rat hepatocytes. *Hepatology* 2000; **32**: 303-311
  - 34 **Critchley JA**, Nimmo GR, Gregson CA, Woolhouse NM, Prescott LF. Inter-subject and ethnic differences in paracetamol metabolism. *Br J Clin Pharmacol* 1986; **22**: 649-657
  - 35 **Tarantino G**, Conca P, Basile V, Gentile A, Capone D, Polichetti G, Leo E. A prospective study of acute drug-induced liver injury in patients suffering from non-alcoholic fatty liver disease. *Hepatol Res* 2007; **37**: 410-415
  - 36 **Parola M**, Robino G. Oxidative stress-related molecules and liver fibrosis. *J Hepatol* 2001; **35**: 297-306
  - 37 **Arteel GE**. Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* 2003; **124**: 778-790
  - 38 **Chen Q**, Cederbaum AI. Cytotoxicity and apoptosis produced by cytochrome P450 2E1 in Hep G2 cells. *Mol Pharmacol* 1998; **53**: 638-648
  - 39 **Konstandi M**, Marselos M, Radon-Camus AM, Johnson E, Lang MA. The role of stress in the regulation of drug metabolizing enzymes in mice. *Eur J Drug Metab Pharmacokinet* 1998; **23**: 483-490
  - 40 **Murray M**. Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis. *Curr Drug Metab* 2006; **7**: 67-81
  - 41 **Ramaiah SK**, Apte U, Mehendale HM. Cytochrome P4502E1 induction increases thioacetamide liver injury in diet-restricted rats. *Drug Metab Dispos* 2001; **29**: 1088-1095
  - 42 **Shakya R**, Rao BS, Shrestha B. Incidence of hepatotoxicity due to antitubercular medicines and assessment of risk factors. *Ann Pharmacother* 2004; **38**: 1074-1079
  - 43 **Eichelbaum M**, Kroemer HK, Mikus G. Genetically determined differences in drug metabolism as a risk factor in drug toxicity. *Toxicol Lett* 1992; **64-65** Spec No: 115-122
  - 44 **Chou WH**, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, Pho M, Xiao V, Ryder TB, Liu WW, Teiling C, Wedlund PJ. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. *J Clin Psychopharmacol* 2000; **20**: 246-251
  - 45 **Scott RJ**, Palmer J, Lewis IA, Pleasance S. Determination of a 'GW cocktail' of cytochrome P450 probe substrates and their metabolites in plasma and urine using automated solid phase extraction and fast gradient liquid chromatography tandem mass spectrometry. *Rapid Commun Mass Spectrom* 1999; **13**: 2305-2319
  - 46 **Yin OQ**, Lam SS, Lo CM, Chow MS. Rapid determination of five probe drugs and their metabolites in human plasma and urine by liquid chromatography/tandem mass spectrometry: application to cytochrome P450 phenotyping studies. *Rapid Commun Mass Spectrom* 2004; **18**: 2921-2933
  - 47 **Mulder AB**, van Lijf HJ, Bon MA, van den Bergh FA, Touw DJ, Neef C, Vermees I. Association of polymorphism in the cytochrome CYP2D6 and the efficacy and tolerability of simvastatin. *Clin Pharmacol Ther* 2001; **70**: 546-551
  - 48 **Kirchheiner J**, Kudlicz D, Meisel C, Bauer S, Meineke I, Roots I, Brockmoller J. Influence of CYP2C9 polymorphisms on the pharmacokinetics and cholesterol-lowering activity of (-)-3S,5R-fluvastatin and (+)-3R,5S-fluvastatin in healthy volunteers. *Clin Pharmacol Ther* 2003; **74**: 186-194
  - 49 **Pachkoria K**, Lucena MI, Ruiz-Cabello F, Crespo E, Cabello MR, Andrade RJ. Genetic polymorphisms of CYP2C9 and CYP2C19 are not related to drug-induced idiosyncratic liver injury (DILI). *Br J Pharmacol* 2007; **150**: 808-815

- 50 **Eichelbaum M**, Evert B. Influence of pharmacogenetics on drug disposition and response. *Clin Exp Pharmacol Physiol* 1996; **23**: 983-985
- 51 **Schwab M**, Schaeffeler E, Klotz U, Treiber G. CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based quadruple therapy for eradication of *Helicobacter pylori*. *Clin Pharmacol Ther* 2004; **76**: 201-209
- 52 **Jia N**, Liu X, Wen J, Qian L, Qian X, Wu Y, Fan G. A proteomic method for analysis of CYP450s protein expression changes in carbon tetrachloride induced male rat liver microsomes. *Toxicology* 2007; **237**: 1-11
- 53 **Geubel AP**, De Galocsy C, Alves N, Rahier J, Dive C. Liver damage caused by therapeutic vitamin A administration: estimate of dose-related toxicity in 41 cases. *Gastroenterology* 1991; **100**: 1701-1709
- 54 **Lammert C**, Einarsson S, Saha C, Niklasson A, Bjornsson E, Chalasani N. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. *Hepatology* 2008; **47**: 2003-2009
- 55 **Fuhr U**. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet* 2000; **38**: 493-504
- 56 **Wang AG**, Xia T, Yuan J, Yu RA, Yang KD, Chen XM, Qu W, Waalkes MP. Effects of phenobarbital on metabolism and toxicity of diclofenac sodium in rat hepatocytes in vitro. *Food Chem Toxicol* 2004; **42**: 1647-1653
- 57 **Somchit N**, Wong CW, Zuraini A, Ahmad Bustamam A, Hasiah AH, Khairi HM, Sulaiman MR, Israf DA. Involvement of phenobarbital and SKF 525A in the hepatotoxicity of antifungal drugs itraconazole and fluconazole in rats. *Drug Chem Toxicol* 2006; **29**: 237-253
- 58 **Mutlib A**, Jiang P, Atherton J, Obert L, Kostrubsky S, Madore S, Nelson S. Identification of potential genomic biomarkers of hepatotoxicity caused by reactive metabolites of N-methylformamide: Application of stable isotope labeled compounds in toxicogenomic studies. *Chem Res Toxicol* 2006; **19**: 1270-1283
- 59 **Chitturi S**, George J. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetic agents, anticonvulsants, lipid-lowering agents, psychotropic drugs. *Semin Liver Dis* 2002; **22**: 169-183
- 60 **Russo MW**, Jacobson IM. How to use statins in patients with chronic liver disease. *Cleve Clin J Med* 2004; **71**: 58-62
- 61 **Ahn BM**. [Herbal preparation-induced liver injury] *Korean J Gastroenterol* 2004; **44**: 113-125
- 62 **Calapai G**, Caputi AP. Herbal medicines: can we do without pharmacologist? *Evid Based Complement Alternat Med* 2007; **4**: 41-43
- 63 **Furbee RB**, Barlotta KS, Allen MK, Holstege CP. Hepatotoxicity associated with herbal products. *Clin Lab Med* 2006; **26**: 227-241, x
- 64 **Stickel F**, Baumuller HM, Seitz K, Vasilakis D, Seitz G, Seitz HK, Schuppan D. Hepatitis induced by Kava (Piper methysticum rhizoma). *J Hepatol* 2003; **39**: 62-67
- 65 **Stickel F**, Poschl G, Seitz HK, Waldherr R, Hahn EG, Schuppan D. Acute hepatitis induced by Greater Celandine (*Chelidonium majus*). *Scand J Gastroenterol* 2003; **38**: 565-568
- 66 **Naranjo CA**, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981; **30**: 239-245
- 67 **Danan G**, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993; **46**: 1323-1330
- 68 **Maria VA**, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997; **26**: 664-669
- 69 **Fick DM**, Cooper JW, Wade WE, Waller JL, Maclean JR, Beers MH. Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. *Arch Intern Med* 2003; **163**: 2716-2724
- 70 **Olivier P**, Caron J, Haramburu F, Imbs JL, Jonville-Bera AP, Lagier G, Sgro C, Vial T, Montastruc JL, Lapeyr-Mestre M. [Validation of a measurement scale: example of a French Adverse Drug Reactions Preventability Scale] *Therapie* 2005; **60**: 39-45
- 71 **Benichou C**, Danan G, Flahault A. Causality assessment of adverse reactions to drugs--II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. *J Clin Epidemiol* 1993; **46**: 1331-1336
- 72 **Arimone Y**, Begaud B, Miremont-Salame G, Fourrier-Reglat A, Moore N, Molimard M, Haramburu F. Agreement of expert judgment in causality assessment of adverse drug reactions. *Eur J Clin Pharmacol* 2005; **61**: 169-173
- 73 **Lancot KL**, Naranjo CA. Comparison of the Bayesian approach and a simple algorithm for assessment of adverse drug events. *Clin Pharmacol Ther* 1995; **58**: 692-698
- 74 **Macedo AF**, Marques FB, Ribeiro CF, Teixeira F. Causality assessment of adverse drug reactions: comparison of the results obtained from published decisional algorithms and from the evaluations of an expert panel. *Pharmacoevidenciol Drug Saf* 2005; **14**: 885-890
- 75 **Knight A**. Systematic reviews of animal experiments demonstrate poor human clinical and toxicological utility. *Altern Lab Anim* 2007; **35**: 641-659
- 76 **Gruber FP**, Dewhurst DG. Alternatives to animal experimentation in biomedical education. *ALTEX* 2004; **21** Suppl 1: 33-48
- 77 **Larson AM**. Acetaminophen hepatotoxicity. *Clin Liver Dis* 2007; **11**: 525-548, vi
- 78 **Rumack BH**. Acetaminophen hepatotoxicity: the first 35 years. *J Toxicol Clin Toxicol* 2002; **40**: 3-20
- 79 **Brass EP**. Hepatic toxicity of antirheumatic drugs. *Cleve Clin J Med* 1993; **60**: 466-472
- 80 **Tanner LA**, Bosco LA, Zimmerman HJ. Hepatic toxicity after acebutolol therapy. *Ann Intern Med* 1989; **111**: 533-534
- 81 **Al-Kawas FH**, Seeff LB, Berendson RA, Zimmerman HJ, Ishak KG. Allopurinol hepatotoxicity. Report of two cases and review of the literature. *Ann Intern Med* 1981; **95**: 588-590
- 82 **Gawlikowski T**, Hydzik P. [Carbamazepine hepatotoxicity--a case report] *Przegl Lek* 2007; **64**: 318-319
- 83 **Hashimoto F**, Davis RL, Egli D. Hepatitis following treatments with famotidine and then cimetidine. *Ann Pharmacother* 1994; **28**: 37-39
- 84 **Wilkinson SP**, Portmann B, Williams R. Hepatitis from dantrolene sodium. *Gut* 1979; **20**: 33-36
- 85 **Tostmann A**, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol* 2008; **23**: 192-202
- 86 **See A**, Hervio P, Bouvry M. [The hepatotoxicity of ethionamide remains a topical subject. Apropos of a case of acute hepatitis] *Ann Gastroenterol Hepatol* (Paris) 1986; **22**: 129-130
- 87 **Sinha A**, Clatch RJ, Stuck G, Blumenthal SA, Patel SA. Isoflurane hepatotoxicity: a case report and review of the literature. *Am J Gastroenterol* 1996; **91**: 2406-2409
- 88 **Robinson DS**, Kurtz NM. What is the degree of risk of hepatotoxicity for depressed patients receiving phenelzine therapy? Is the risk sufficient to require that we modify the written advice (as to diet and risks) that we regularly give our patients before we institute this therapy? *J Clin Psychopharmacol* 1987; **7**: 61-62
- 89 **Portal RW**, Emanuel RW. Phenindione hepatitis complicating anticoagulant therapy. *Br Med J* 1961; **2**: 1318-1319
- 90 **Roberts EA**, Spielberg SP, Goldbach M, Phillips MJ. Phenobarbital hepatotoxicity in an 8-month-old infant. *J Hepatol* 1990; **10**: 235-239
- 91 **Brackett CC**, Bloch JD. Phenytoin as a possible cause of acetaminophen hepatotoxicity: case report and review of the

- literature. *Pharmacotherapy* 2000; **20**: 229-233
- 92 **Ray DC**, Drummond GB. Halothane hepatitis. *Br J Anaesth* 1991; **67**: 84-99
  - 93 **Lake-Bakaar G**, Scheuer PJ, Sherlock S. Hepatic reactions associated with ketoconazole in the United Kingdom. *Br Med J (Clin Res Ed)* 1987; **294**: 419-422
  - 94 **Clark JA**, Zimmerman HJ, Tanner LA. Labetalol hepatotoxicity. *Ann Intern Med* 1990; **113**: 210-213
  - 95 **Weinstein RP**, Gosselin JY. Case report of hepatotoxicity associated with maprotiline. *Can J Psychiatry* 1988; **33**: 233-234
  - 96 **Lennard MS**. Metoprolol-induced hepatitis: is the rate of oxidation related to drug-induced hepatotoxicity? *Hepatology* 1989; **9**: 163-164
  - 97 **Barbare JC**, Biour M, Cadot T, Latrive JP. [Hepatotoxicity of mianserin: a case with positive reintroduction] *Gastroenterol Clin Biol* 1992; **16**: 486-488
  - 98 **Poli M**, Cordie L. [Liver disease caused by PAS: toxic manifestations of PAS.] *Arch Sci Med (Torino)* 1952; **93**: 391-424
  - 99 **Handl SD**, Hirsch NR, Haas K, Davidson FZ. Quinidine hepatitis. *Arch Intern Med* 1975; **135**: 871-872
  - 100 **Larrey D**, Vial T, Micaleff A, Babany G, Morichau-Beauchant M, Michel H, Benhamou JP. Hepatitis associated with amoxycillin-clavulanic acid combination report of 15 cases. *Gut* 1992; **33**: 368-371
  - 101 **Ribeiro JM**, Lucas M, Baptista A, Victorino RM. Fatal hepatitis associated with ranitidine. *Am J Gastroenterol* 2000; **95**: 559-560
  - 102 **Mainra RR**, Card SE. Trimethoprim-sulfamethoxazole-associated hepatotoxicity - part of a hypersensitivity syndrome. *Can J Clin Pharmacol* 2003; **10**: 175-178
  - 103 **Lucena MI**, Carvajal A, Andrade RJ, Velasco A. Antidepressant-induced hepatotoxicity. *Expert Opin Drug Saf* 2003; **2**: 249-262
  - 104 **Zaman F**, Ye G, Abreo KD, Latif S, Zibari GB. Successful orthotopic liver transplantation after trimethoprim-sulfamethoxazole associated fulminant liver failure. *Clin Transplant* 2003; **17**: 461-464
  - 105 **Tennison MB**, Miles MV, Pollack GM, Thorn MD, Dupuis RE. Valproate metabolites and hepatotoxicity in an epileptic population. *Epilepsia* 1988; **29**: 543-547
  - 106 **Odeh M**, Oliven A. [Verapamil-associated liver injury] *Harefuah* 1998; **134**: 36-37
  - 107 **Dourakis SP**, Sevastianov VA, Kaliopi P. Acute severe steatohepatitis related to prednisolone therapy. *Am J Gastroenterol* 2002; **97**: 1074-1075
  - 108 **de Leval L**, Lambermont B, D'Orio V, Boniver J. Fatal massive liver steatosis--a clinicopathological case report. *Acta Gastroenterol Belg* 1997; **60**: 180-183
  - 109 **Berson A**, De Beco V, Letteron P, Robin MA, Moreau C, El Kahwaji J, Verthier N, Feldmann G, Fromenty B, Pessayre D. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. *Gastroenterology* 1998; **114**: 764-774
  - 110 **Pratt CB**, Johnson WW. Duration and severity of fatty metamorphosis of the liver following L-asparaginase therapy. *Cancer* 1971; **28**: 361-364
  - 111 **Gradon JD**, Sepkowitz DV. Massive hepatic enlargement with fatty change associated with ketoconazole. *DICP* 1990; **24**: 1175-1176
  - 112 **Arranto AJ**, Sotaniemi EA. Morphologic alterations in patients with alpha-methyl-dopa-induced liver damage after short- and long-term exposure. *Scand J Gastroenterol* 1981; **16**: 853-863
  - 113 **Babany G**, Uzzan F, Larrey D, Degott C, Bourgeois P, Rene E, Vissuzaine C, Erlinger S, Benhamou JP. Alcoholic-like liver lesions induced by nifedipine. *J Hepatol* 1989; **9**: 252-255
  - 114 **Deschamps D**, DeBeco V, Fisch C, Fromenty B, Guillouzo A, Pessayre D. Inhibition by perhexiline of oxidative phosphorylation and the beta-oxidation of fatty acids: possible role in pseudoalcoholic liver lesions. *Hepatology* 1994; **19**: 948-961
  - 115 **Wenk RE**, Gebhardt FC, Bhagavan BS, Lustgarten JA, McCarthy EF. Tetracycline-associated fatty liver of pregnancy, including possible pregnancy risk after chronic dermatologic use of tetracycline. *J Reprod Med* 1981; **26**: 135-141
  - 116 **Walia KS**, Khan EA, Ko DH, Raza SS, Khan YN. Side effects of antiepileptics--a review. *Pain Pract* 2004; **4**: 194-203
  - 117 **Bienvenu L**, Burel F, Hofman V, Itchai C, Amaro J, Hofman P. [A rare etiology of hepatic steatosis associated with lactic acidosis: the toxicity of antiviral nucleoside analogues] *Ann Pathol* 2001; **21**: 160-163
  - 118 **Alexander P**, Roskams T, Van Steenberghe W, Peetermans W, Desmet V, Yap SH. Intrahepatic cholestasis induced by amoxicillin/clavulanic acid (Augmentin): a report on two cases. *Acta Clin Belg* 1991; **46**: 327-332
  - 119 **Eisenbach C**, Goeggelmann C, Flechtenmacher C, Stremmel W, Encke J. Severe cholestatic hepatitis caused by azathioprine. *Immunopharmacol Immunotoxicol* 2005; **27**: 77-83
  - 120 **Schattnr A**, Kozak N, Friedman J. Captopril-induced jaundice: report of 2 cases and a review of 13 additional reports in the literature. *Am J Med Sci* 2001; **322**: 236-240
  - 121 **Larrey D**, Hadengue A, Pessayre D, Choudat L, DeGott C, Benhamou JP. Carbamazepine-induced acute cholangitis. *Dig Dis Sci* 1987; **32**: 554-557
  - 122 **Chan AO**, Ng IO, Lam CM, Shek TW, Lai CL. Cholestatic jaundice caused by sequential carbimazole and propylthiouracil treatment for thyrotoxicosis. *Hong Kong Med J* 2003; **9**: 377-380
  - 123 **Skoog SM**, Smyrk TC, Talwalkar JA. Cephalixin-induced cholestatic hepatitis. *J Clin Gastroenterol* 2004; **38**: 833
  - 124 **Lo KJ**, Eastwood IR, Eidelman S. Cholestatic jaundice associated with chlorthalidone hydrochloride (Librium) therapy. Report of a case and review of the literature. *Am J Dig Dis* 1967; **12**: 845-849
  - 125 **Gupta R**, Sachar DB. Chlorpropamide-induced cholestatic jaundice and pseudomembranous colitis. *Am J Gastroenterol* 1985; **80**: 381-383
  - 126 **Lotric S**, Lejko-Zupanc T, Jereb M. Cloxacillin-induced cholestasis. *Clin Infect Dis* 1994; **19**: 981-982
  - 127 **Day C**, Hewins P, Sheikh L, Kilby M, McPake D, Lipkin G. Cholestasis in pregnancy associated with ciclosporin therapy in renal transplant recipients. *Transpl Int* 2006; **19**: 1026-1029
  - 128 **Silva MO**, Reddy KR, McDonald T, Jeffers LJ, Schiff ER. Danazol-induced cholestasis. *Am J Gastroenterol* 1989; **84**: 426-428
  - 129 **Bakris GL**, Cross PD, Hammarsten JE. Disopyramide-associated liver dysfunction. *Mayo Clin Proc* 1983; **58**: 265-267
  - 130 **Todd P**, Levison D, Farthing MJ. Enalapril-related cholestatic jaundice. *J R Soc Med* 1990; **83**: 271-272
  - 131 **Derby LE**, Jick H, Henry DA, Dean AD. Erythromycin-associated cholestatic hepatitis. *Med J Aust* 1993; **158**: 600-602
  - 132 **Devereaux BM**, Crawford DH, Purcell P, Powell LW, Roser HP. Flucloxacillin associated cholestatic hepatitis. An Australian and Swedish epidemic? *Eur J Clin Pharmacol* 1995; **49**: 81-85
  - 133 **Reynolds R**, Lloyd DA, Slinger RP. Cholestatic jaundice induced by flurazepam hydrochloride. *Can Med Assoc J* 1981; **124**: 893-894
  - 134 **Lee HW**, Chung JP, Lee KS, Kim KC, Lee KS, Chon CY, Park IS, Kim HG. A case of flutamide-induced acute cholestatic hepatitis--a case report. *Yonsei Med J* 1996; **37**: 225-229
  - 135 **Basset C**, Vadrot J, Denis J, Poupon J, Zafrani ES. Prolonged cholestasis and ductopenia following gold salt therapy. *Liver Int* 2003; **23**: 89-93
  - 136 **Chiprut RO**, Viteri A, Jamroz C, Dyck WP. Intrahepatic cholestasis after griseofulvin administration. *Gastroenterology* 1976; **70**: 1141-1143
  - 137 **van Basten JP**, van Hoek B, Zeijen R, Stockbrugger R. Glyburide-induced cholestatic hepatitis and liver failure. Case-report and review of the literature. *Neth J Med* 1992; **40**: 305-307
  - 138 **Horst DA**, Grace ND, LeCompte PM. Prolonged cholestasis and progressive hepatic fibrosis following imipramine

- therapy. *Gastroenterology* 1980; **79**: 550-554
- 139 **Dincsoy HP**, Saelinger DA. Haloperidol-induced chronic cholestatic liver disease. *Gastroenterology* 1982; **83**: 694-700
  - 140 **Benson GD**, Anderson PK, Combes B, Ishak KG. Prolonged jaundice following ketoconazole-induced hepatic injury. *Dig Dis Sci* 1988; **33**: 240-246
  - 141 **Foitzl DR**, Hyman G, Lefkowitz JH. Jaundice and intrahepatic cholestasis following high-dose megestrol acetate for breast cancer. *Cancer* 1989; **63**: 438-439
  - 142 **Schmidt G**, Borsch G, Muller KM, Wegener M. Methimazole-associated cholestatic liver injury: case report and brief literature review. *Hepatogastroenterology* 1986; **33**: 244-246
  - 143 **Lucey MR**, Moseley RH. Severe cholestasis associated with methyltestosterone: a case report. *Am J Gastroenterol* 1987; **82**: 461-462
  - 144 **Kiire CF**, Rutherford D. Nifedipine-associated jaundice: a second case. *East Afr Med J* 1986; **63**: 560-561
  - 145 **Mulberg AE**, Bell LM. Fatal cholestatic hepatitis and multi-system failure associated with nitrofurantoin. *J Pediatr Gastroenterol Nutr* 1993; **17**: 307-309
  - 146 **Gilbert EF**, Dasilva AQ, Queen DM. Intrahepatic cholestasis with fatal termination following norethandrolone therapy. *JAMA* 1963; **185**: 538-539
  - 147 **Lieberman DA**, Keeffe EB, Stenzel P. Severe and prolonged oral contraceptive jaundice. *J Clin Gastroenterol* 1984; **6**: 145-148
  - 148 **Moradpour D**, Altorfer J, Flury R, Greminger P, Meyenberger C, Jost R, Schmid M. Chlorpromazine-induced vanishing bile duct syndrome leading to biliary cirrhosis. *Hepatology* 1994; **20**: 1437-1441
  - 149 **Altuntas Y**, Ozturk B, Erdem L, Gunes G, Karul S, Ucak S, Sengul A. Phenytoin-induced toxic cholestatic hepatitis in a patient with skin lesions: case report. *South Med J* 2003; **96**: 201-203
  - 150 **Gefel D**, Harats N, Lijovetsky G, Eliakim M. Cholestatic jaundice associated with D-penicillamine therapy. *Scand J Rheumatol* 1985; **14**: 303-306
  - 151 **Rosenberg WM**, Ryley NG, Trowell JM, McGee JO, Chapman RW. Dextropropoxyphene induced hepatotoxicity: a report of nine cases. *J Hepatol* 1993; **19**: 470-474
  - 152 **Kouklakis G**, Mpoumpoumaris A, Zazos P, Moschos J, Koulaouzidis A, Nakos A, Pehlivanidis A, Iosiphidis M, Molyvas E, Nikolaidis N. Cholestatic hepatitis with severe systemic reactions induced by trimethoprim-sulfamethoxazole. *Ann Hepatol* 2007; **6**: 63-65
  - 153 **Lasso De La Vega MC**, Zapater P, Such J, Sola-Vera J, Paya A, Horga JF, Perez-Mateo M. [Toxic hepatitis associated with tamoxifen use. A case report and literature review] *Gastroenterol Hepatol* 2002; **25**: 247-250
  - 154 **Eland IA**, Kerkhof SC, Overbosch D, Wismans PJ, Stricker BH. [Cholestatic hepatitis ascribed to the use of thiabendazole] *Ned Tijdschr Geneesk* 1998; **142**: 1331-1334
  - 155 **Nakao NL**, Gelb AM, Stenger RJ, Siegel JH. A case of chronic liver disease due to tolazamide. *Gastroenterology* 1985; **89**: 192-195
  - 156 **Randeva HS**, Bangar V, Sailesh S, Hillhouse EW. Fatal cholestatic jaundice associated with amitriptyline. *Int J Clin Pract* 2000; **54**: 405-406
  - 157 **Larrey D**, Amouyal G, Danan G, Degott C, Pessayre D, Benhamou JP. Prolonged cholestasis after troleandomycin-induced acute hepatitis. *J Hepatol* 1987; **4**: 327-329
  - 158 **Burgunder JM**, Abernethy DR, Lauterburg BH. Liver injury due to verapamil. *Hepatogastroenterology* 1988; **35**: 169-170
  - 159 **Vanderstigel M**, Zafrani ES, Lejone JL, Schaeffer A, Portos JL. Allopurinol hypersensitivity syndrome as a cause of hepatic fibrin-ring granulomas. *Gastroenterology* 1986; **90**: 188-190
  - 160 **Levy M**, Goodman MW, Van Dyne BJ, Sumner HW. Granulomatous hepatitis secondary to carbamazepine. *Ann Intern Med* 1981; **95**: 64-65
  - 161 **Ben-Yehuda A**, Bloom A, Lijovetsky G, Flusser D, Tur-Kaspa R. Chlorpromazine-induced liver and bone marrow granulomas associated with agranulocytosis. *Isr J Med Sci* 1990; **26**: 449-451
  - 162 **Sarachek NS**, London RL, Matulewicz TJ. Diltiazem and granulomatous hepatitis. *Gastroenterology* 1985; **88**: 1260-1262
  - 163 **Harats N**, Ehrenfeld M, Shalit M, Lijovetsky G. Gold-induced granulomatous hepatitis. *Isr J Med Sci* 1985; **21**: 753-756
  - 164 **Jori GP**, Peschile C. Hydralazine disease associated with transient granulomas in the liver. A case report. *Gastroenterology* 1973; **64**: 1163-1167
  - 165 **Sippel PJ**, Agger WA. Nitrofurantoin-induced granulomatous hepatitis. *Urology* 1981; **18**: 177-178
  - 166 **Silvain C**, Fort E, Levillain P, Labat-Labourdette J, Beauchant M. Granulomatous hepatitis due to combination of amoxicillin and clavulanic acid. *Dig Dis Sci* 1992; **37**: 150-152
  - 167 **Cook IF**, Shilkin KB, Reed WD. Phenytoin induced granulomatous hepatitis. *Aust N Z J Med* 1981; **11**: 539-541
  - 168 **Knobel B**, Buyanowsky G, Dan M, Zaidel L. Pyrazinamide-induced granulomatous hepatitis. *J Clin Gastroenterol* 1997; **24**: 264-266
  - 169 **Bramlet DA**, Posalaky Z, Olson R. Granulomatous hepatitis as a manifestation of quinidine hypersensitivity. *Arch Intern Med* 1980; **140**: 395-397
  - 170 **Namias A**, Bhalotra R, Donowitz M. Reversible sulfasalazine-induced granulomatous hepatitis. *J Clin Gastroenterol* 1981; **3**: 193-198
  - 171 **Seeff LB**. Drug-induced chronic liver disease, with emphasis on chronic active hepatitis. *Semin Liver Dis* 1981; **1**: 104-115
  - 172 **Black M**, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. *Gastroenterology* 1975; **69**: 289-302
  - 173 **Balazs M**, Kovach G. Chronic aggressive hepatitis after methyl dopa treatment. Case report with electron-microscopic study. *Hepatogastroenterology* 1981; **28**: 199-202
  - 174 **Roy AK**, Mahoney HC, Levine RA. Phenytoin-induced chronic hepatitis. *Dig Dis Sci* 1993; **38**: 740-743
  - 175 **Aponte J**, Petrelli M. Histopathologic findings in the liver of rheumatoid arthritis patients treated with long-term bolus methotrexate. *Arthritis Rheum* 1988; **31**: 1457-1464
  - 176 **Volbeda F**, Jonker AM, Vecht J, Groeneveld PH. [Liver cirrhosis due to chronic use of nitrofurantoin] *Ned Tijdschr Geneesk* 2004; **148**: 235-238
  - 177 **Anania FA**, Rabin L. Terbinafine hepatotoxicity resulting in chronic biliary ductopenia and portal fibrosis. *Am J Med* 2002; **112**: 741-742
  - 178 **Giannitrapani L**, Soresi M, La Spada E, Cervello M, D'Alessandro N, Montalto G. Sex hormones and risk of liver tumor. *Ann N Y Acad Sci* 2006; **1089**: 228-236
  - 179 **Bartley J**, Loddenkemper C, Lange J, Mechsner S, Radke C, Neuhaus P, Ebert AD. Hepatocellular adenoma and focal nodular hyperplasia after long-term use of danazol for endometriosis: a case report. *Arch Gynecol Obstet* 2004; **269**: 290-293
  - 180 **Zhu AX**, Lauwers GY, Tanabe KK. Cholangiocarcinoma in association with Thorotrast exposure. *J Hepatobiliary Pancreat Surg* 2004; **11**: 430-433
  - 181 **Chopra S**, Edelstein A, Koff RS, Zimelman AP, Lacson A, Neiman RS. Peliosis hepatis in hematologic disease. Report of two cases. *JAMA* 1978; **240**: 1153-1155
  - 182 **Sebagh M**, Debette M, Samuel D, Emile JF, Falissard B, Cailiez V, Shouval D, Bismuth H, Reynes M. "Silent" presentation of veno-occlusive disease after liver transplantation as part of the process of cellular rejection with endothelial predilection. *Hepatology* 1999; **30**: 1144-1150
  - 183 **Essell JH**, Thompson JM, Harman GS, Halvorson RD, Snyder MJ, Johnson RA, Rubinsak JR. Marked increase in veno-occlusive disease of the liver associated with methotrexate use for graft-versus-host disease prophylaxis in patients receiving busulfan/cyclophosphamide. *Blood* 1992; **79**: 2784-2788



- 184 **Voigt H**, Caselitz J, Janner M. [Veno-occlusive syndrome with acute liver dystrophy following decarbazine therapy of malignant melanoma (author's transl)] *Klin Wochenschr* 1981; **59**: 229-236
- 185 **Spormann H**, Willgeroth C, Tautenhahn P. [Peliosis hepatis with liver rupture] *Zentralbl Allg Pathol* 1985; **130**: 545-550
- 186 **Lennard L**, Richards S, Cartwright CS, Mitchell C, Lilleyman JS, Vora A. The thiopurine methyltransferase genetic polymorphism is associated with thioguanine-related veno-occlusive disease of the liver in children with acute lymphoblastic leukemia. *Clin Pharmacol Ther* 2006; **80**: 375-383
- 187 **Sulis ML**, Bessmertry O, Granowetter L, Weiner M, Kelly KM. Veno-occlusive disease in pediatric patients receiving actinomycin D and vincristine only for the treatment of rhabdomyosarcoma. *J Pediatr Hematol Oncol* 2004; **26**: 843-846
- 188 **Ouellet G**, Tremblay L, Marleau D. Fulminant hepatitis induced by lamotrigine. *South Med J* 2009; **102**: 82-84
- 189 **Tan HH**, Ong WM, Lai SH, Chow WC. Nimesulide-induced hepatotoxicity and fatal hepatic failure. *Singapore Med J* 2007; **48**: 582-585
- 190 **Skopp G**, Schmitt HP, Pedal I. [Fulminant liver failure in a patient on carbamazepine and levetiracetam treatment associated with status epilepticus] *Arch Kriminol* 2006; **217**: 161-175
- 191 **Barcena R**, Oton E, Angeles Moreno M, Fortun J, Garcia-Gonzalez M, Moreno A, de Vicente E. Is liver transplantation advisable for isoniazid fulminant hepatitis in active extrapulmonary tuberculosis? *Am J Transplant* 2005; **5**: 2796-2798
- 192 **Tietz A**, Heim MH, Eriksson U, Marsch S, Terracciano L, Krahenbuhl S. Fulminant liver failure associated with clarithromycin. *Ann Pharmacother* 2003; **37**: 57-60
- 193 **Garbino J**, Henry JA, Mentha G, Romand JA. Ecstasy ingestion and fulminant hepatic failure: liver transplantation to be considered as a last therapeutic option. *Vet Hum Toxicol* 2001; **43**: 99-102
- 194 **Stickel F**, Egerer G, Seitz HK. Hepatotoxicity of botanicals. *Public Health Nutr* 2000; **3**: 113-124
- 195 **Gordon DW**, Rosenthal G, Hart J, Sirota R, Baker AL. Chaparral ingestion. The broadening spectrum of liver injury caused by herbal medications. *JAMA* 1995; **273**: 489-490
- 196 **Russmann S**, Lauterburg BH, Helbling A. Kava hepatotoxicity. *Ann Intern Med* 2001; **135**: 68-69
- 197 **Lee MK**, Cheng BW, Che CT, Hsieh DP. Cytotoxicity assessment of Ma-huang (Ephedra) under different conditions of preparation. *Toxicol Sci* 2000; **56**: 424-430
- 198 **Whiting PW**, Clouston A, Kerlin P. Black cohosh and other herbal remedies associated with acute hepatitis. *Med J Aust* 2002; **177**: 440-443
- 199 **Lake CR**, Gallant S, Masson E, Miller P. Adverse drug effects attributed to phenylpropanolamine: a review of 142 case reports. *Am J Med* 1990; **89**: 195-208
- 200 **Sanchez W**, Maple JT, Burgart LJ, Kamath PS. Severe hepatotoxicity associated with use of a dietary supplement containing usnic acid. *Mayo Clin Proc* 2006; **81**: 541-544
- 201 **Bleibel W**, Kim S, D'Silva K, Lemmer ER. Drug-induced liver injury: review article. *Dig Dis Sci* 2007; **52**: 2463-2471

S- Editor Li LF L- Editor O'Neill M E- Editor Ma WH