Idiosyncratic Drug Hepatotoxicity

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IDIOSYNCRATIC DRUG HEPATOTOXICITY

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Abstract | The occurrence of idiosyncratic drug hepatotoxicity is a major problem in all phases of clinical drug development and the most frequent cause of post-marketing warnings and withdrawals. This review examines the clinical signatures of this problem, signals predictive of its occurrence (particularly of more frequent, reversible, low-grade injury) and the role of monitoring in prevention by examining several recent examples (for example, troglitazone). In addition, the failure of preclinical toxicology to predict idiosyncratic reactions, and what can be done to improve this problem, is discussed. Finally, our current understanding of the pathophysiology of experimental drug hepatotoxicity is examined, focusing on acetaminophen, particularly with respect to the role of the innate immune system and control of cell-death pathways, which might provide targets for exploration and identification of risk factors and mechanisms in humans.

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Drug-induced liver disease (DILD) represents a major challenge for clinicians, the pharmaceutical industry and regulatory agencies worldwide, including the FDA. The most catastrophic consequence is the occurrence of acute liver failure that leads to death or requires liver transplantation. DILD is the leading cause of acute liver failure in the United States, accounting for about half of all cases[1]. Although acetaminophen (APAP; paracetamol in the United Kingdom) poisoning (roughly equally divided between intentional and unintentional overdose) accounts for the bulk of drug-related cases, more than 10% of cases of acute liver failure are due to idiosyncratic hepatotoxicity. Drug hepatotoxicity is also a leading cause of failures in drug development at the clinical phases of investigation[2]. It is remarkable that in most instances routine animal toxicology fails to identify the risk of subsequent problems in clinical stages of drug development or to predict post-marketing problems. The post-marketing occurrence of drug hepatotoxicity is a leading cause of regulatory actions, which include drug withdrawals, modifications of use and warnings (TABLE 1). In addition to pharmaceuticals, herbal remedies and dietary supplements are increasingly being recognized as causes of idiosyncratic hepatotoxicity. Due to limitations of space, the present discussion will be restricted to pharmaceuticals.

Allergic versus non-allergic idiosyncrasy

Idiosyncrasy signifies the uniqueness of the individual and by definition idiosyncratic drug hepatotoxicity occurs in only a small proportion of individuals exposed to a drug. The key question in the field is, 'what accounts for the uniqueness of the occasional individual who is adversely affected?'. Although the answer remains elusive, it seems unquestionable that genetic and environmental factors are involved.

Idiosyncratic reactions can be classified as allergic or non-allergic. Allergic reactions involve the participation of the adaptive immune system[3]. In some parts of the world, lymphocyte-stimulation tests are used to identify this mechanism. The test involves exposure of peripheral blood mononuclear cells from the patient to the drug, and subsequent determination of lymphocyte proliferation using radiolabelled thymidine incorporation measured in the presence of a prostaglandin inhibitor to block participation of suppressor cells[4,5]. This approach has not gained favour in the United States, perhaps because of the lack of standardization and reproducibility. Nevertheless, because of the promising results of this test in the diagnosis of allergic hepatotoxicity, a concerted, global effort to validate and test this approach should be encouraged. Identification of the allergic idiosyncratic reaction is

Table 1 | **Regulatory actions due to non-allergic hepatotoxicity***

Drug	Use	Regulatory action
Bromfenac	Analgesic	Withdrawn
Troglitazone	Diabetes	Withdrawn
Felbamate	Anticonvulsant	Restricted use
Pemoline	CNS stimulant	Restricted use
Tolcapone	Parkinson's disease	Restricted use
Trovafloxacin	Antibiotic	Restricted use
Acetaminophen	Analgesic	Warnings
Leflunomide	Immunomodulator	Warnings
Nefazodone	Antipsychotic	Warnings
Nevirapine	Antiviral (HIV)	Warnings
Pyrazinamide	Antituberculosis	Warnings
Rifampin	Antituberculosis	Warnings
Terbinafine	Antifungal	Warnings
Valproic acid	Anticonvulsant	Warnings
Zafirlukast	Asthma	Warnings

*In the past decade; excludes some cases associated with nevirapine (allergic hepatotoxicity). Adapted with permission from Paul Watkins. CNS, central nervous system.

currently a circumstantial diagnosis. The characteristics include the presence of fever, rash, eosinophilia, a relatively short latency (usually one month or less), the presence of autoantibodies (for example, antinuclear antibodies) and the rapid recurrence of hepatotoxicity on re-exposure to the drug[6]. The allergic basis for the pathogenesis of an idiosyncratic hepatotoxic reaction is strongly supported by the occurrence of all of these features; however, often only some are present and in a variable proportion of affected patients. It is therefore conceivable that any drug could cause either allergic or non-allergic toxicity. TABLES 2,3 list examples of drugs classified as allergic and non-allergic, respectively. A common belief is that idiosyncratic allergic hepatotoxicity is unrelated to dose. This is a misconception for the following reasons. First, these reactions are very rare (if they occur at all) when the dose of any drug is less than 10 mg per day[7]. Second, allergic reactions occur more frequently at higher doses or with more frequent exposure (for example, halothane)[8]. Third, the immunological phenomenon of desensitization points to the requirement of dose threshold. Unfortunately, animal models of allergic hepatotoxicity have not been developed, and is therefore an area in need of investigation.

The non-allergic idiosyncratic reactions are characterized by the consistent absence of these features of hypersensitivity, but it is not possible to entirely exclude an allergic mechanism on the basis of absence of these features. The conclusion that an idiosyncratic reaction is not allergic must therefore be viewed as tentative. Nevertheless, some features support the validity of the distinction between allergic and non-allergic reactions. A very important feature is the long latency period of many months observed in non-allergic idiosyncratic reactions[2,6]. Patients can have normal liver test results for 6 months and then suddenly develop hepatotoxicity. This is a very puzzling scenario, particularly when the pharmacokinetic properties of a drug exclude accumulation of the drug in the liver as an explanation; amiodarone is an example of such an accumulation-related injury[9]. Non-allergic hepatotoxicity can be apparently independent of dose (for example, troglitazone, discussed later)[10] or be dose-related (for example, statins)[11]. Furthermore, rechallenge after resolution of the non-allergic injury might not consistently reproduce the injury, indicating that some factors in the environment at the time of the original injury are no longer present or that some type of adaptation has occurred.

Low-grade toxicity and the danger hypothesis

A phenomenon common to both types of rare idiosyncratic reaction is a background of more frequent, but mild, and often transient, asymptomatic liver injury. This might signal an individual's vulnerability to further injury, and its transient nature indicates that some type of adaptation usually occurs. Although it is uncertain whether the mechanism of mild injury determines the likelihood of more severe injury, it is possible that the concomitant contribution of genetic and environmental factors to an initial injury, as well as individual deficiencies in the adaptive processes that limit the extent of the injury, could lead to unopposed injury progression.

In considering allergic drug reactions, it is useful to draw from the concepts of autoimmune diseases and reactions. In this regard, it has recently been proposed that autoimmunity (when the immune system loses normal tolerance of self and reacts against self) is allowed to develop if it is also responding to some type of danger. The danger hypothesis[12] can be adapted to allergic drug hepatotoxicity[7]. If a drug is metabolized in the liver to form a reactive compound that covalently binds to proteins (haptenization), this alone might be insufficient to trigger an immune reaction or could induce a non-pathogenic immune response (for example, the common occurrence of anti-CYP2E1 antibodies in halothane-exposed anaesthesiologists)[13]. For the development of an immune response to the HAPTEN, a second co-stimulatory trigger is proposed — that is, a so-called 'danger' signal (FIG. 1). The danger that primes a genetically susceptible adaptive immune system might include the background mild hepatic injury discussed above or concomitant infection or inflammatory conditions, such as HIV, or other viral or bacterial infections. For example, allergic hepatotoxicity is more common in AIDS patients[14]. As discussed below, the altered cytokine milieu of chronic viral disease can also influence susceptibility to non-allergic toxicity and helps to explain the suggested increased susceptibility of patients with HIV or chronic HEPATITIS B and C to isoniazid hepatotoxicity[15–17]. Although clearly speculative at present, experimental support for such a mechanism has begun to emerge (see below section on APAP toxicity) and this will be a fruitful area for

HAPTEN
A small molecule that reacts with a specific antibody but which cannot induce the formation of antibodies unless bound to a carrier protein or other large antigenic molecule.

Table 2 | **Idiosyncratic allergic hepatotoxins in current use**

Drug	Indication/drug action
Hepatocellular injury	
Allopurinol	Gout
Diclofenac*	Analgesic
Dihydralazine	Antihypertensive
Halothane	Anaesthetic
Methyldopa	Antihypertensive
Minocycline	Antibiotic
Nitrofurantoin	Antibiotic
Phenytoin	Anticonvulsant
Propylthiouracil	Antithyroid
Cholestatic injury	
Angiotensin-converting enzyme inhibitors	Hypertension
Amoxicillin–clavulanic acid	Antibiotic
Phenothiazines	Antipsychotic
Erythromycins	Antibiotic
Sulindac	Analgesic
Tricyclic antidepressants	Depression

*Elicits allergic and non-allergic mechanisms.

further basic and clinical investigation. Additionally, genetic polymorphisms that determine the response of the adaptive immune system might be important. Although a number of studies of human leukocyte antigen (HLA) polymorphisms have been performed in populations exhibiting allergic idiosyncratic toxicities that demonstrate associations, most have revealed drug-specific associations that are not generalized and therefore do not provide universally applicable mechanistic clues about the nature of drug-induced injury (except for cholestatic reactions discussed below) or have provided negative results.

Signals of toxicity in clinical trials

Another aspect of the phenomenon of mild background injury relates to the interpretation of signals in clinical trials during drug development. Nearly all the recent examples of non-allergic idiosyncratic hepatotoxicity have been accompanied by an increased frequency of serum alanine aminotransferase (ALT) abnormalities in clinical trials. Increased serum ALT is almost always a consequence of release by dead or dying hepatocytes, and is a sensitive semi-quantitative measure of liver injury. Occasionally serum ALT levels are elevated in muscle injury, which can be recognized by concomitant disproportionately high levels of muscle enzymes in serum. As a general rule, ALT levels greater than three times the upper limits of normal (ULN) has been somewhat arbitrarily identified as a sensitive, but not necessarily specific, signal for liver toxicity. Three times ULN in the absence of an increase in serum bilirubin reflects very mild injury, so it is reasonable to focus on this level. Background rates of serum ALT greater than three times ULN in placebo-treated patients depend somewhat on the disease being studied but are generally low, in the range 0.2–1.0%. A statistically significant doubling of the incidence of serum ALT greater than three times ULN is nearly universally described with idiosyncratic hepatotoxins. However, this finding is not always predictive of more severe outcomes — that is, overt idiosyncratic toxicity (symptoms and jaundice). For example, although most statins are associated with a dose-related increase in the incidence of ALT greater than three times ULN, acute liver failure occurs in about one in a million treated patients, an incidence no greater than the estimated incidence of idiopathic acute liver failure[11]. Nevertheless, although statins seem to be very safe at low and intermediate doses, the continual lowering of the serum cholesterol/low-density lipoprotein (LDL) bar, increasing doses and the arrival of new and more potent statins does raise some concerns, because the incidence of overt hepatic injury with jaundice could increase to one per thousand[18], and acute liver failure is predicted to occur in some of these cases. Indeed, the statins represent a rare example in which a pharmacodynamic property of a drug class accounts for its toxicity. Animal models with low expression of 3-hydroxy-3-methyl glutaryl co-enzyme A (HMG-CoA) reductase, the target for statin inhibition, were more susceptible to injury, which could be rescued with treatment with the deficient enzyme product, mevalonic acid[19,20].

Considering the problems with lack of specificity of three times ULN ALT as a signal for severe liver toxicity, it is important to look for evidence of more severe injury during clinical drug development. Acute liver failure, although sometimes encountered in clinical trials (for example, recent experience with ximelagatran, discussed in more detail later), is unlikely to occur in a population of several thousand clinical trial patients when the incidence of acute liver failure is in the range of 1 in 10,000 or less[21]. It is therefore necessary to look at other milestones, such as increased incidence of ALT greater than ten times ULN (rarely encountered in placebo-treated patients) and ALT greater than three times ULN accompanied by hyperbilirubinaemia (excluding biliary obstruction or Gilbert's syndrome-unconjugated hyperbilirubinaemia). Some confusion exists about the bilirubin level of concern because the FDA has recently focused on serum bilirubin two times ULN as a predictor of more severe toxicity, whereas the threshold for the onset of jaundice is greater than three times ULN. Many years ago Zimmerman noted that elevated serum transaminases accompanied by jaundice due to drug toxicity was associated with a mortality of ~10% (range 5–50%)[22]. This has been referred to as Hy's law and has withstood the test of time. In cases in which hepatocellular injury accompanied by jaundice occurs in clinical trials, the FDA has adapted Zimmerman's observations and refers to these as 'Hy's law' cases because they can be predictive of more severe acute liver failure. However, the predictability of the incidence of life-threatening injury when bilirubin increases to 2–3 mg per dl (below the jaundice threshold implicit in Hy's law) is less firmly established, although clearly of

HEPATITIS
Inflammation of the liver, caused by infectious or toxic agents and characterized by jaundice, fever, liver enlargement and abdominal pain.

Table 3 | **Idiosyncratic non-allergic hepatotoxins in current use**

Drug	Indication/drug action
Hepatocellular injury	
Acarbose	Diabetes
Amiodarone	Anti-arrhythmic
Bosentan	Pulmonary hypertension
Dantrolene	Muscle relaxant
Diclofenac*	Analgesic
Disulphiram	Alcoholism
Felbamate	Anticonvulsant
Flutamide	Anti-androgen
Isoniazid	Antituberculosis
Isotretinoin	Acne
Ketoconazole	Antifungal
Labetalol	Antihypertensive
Leflunomide	Immunomodulator
Nefazodone	Antipsychotic
Niacin	Cholesterol-lowering
Pemoline	CNS stimulant
Pyrazinamide (± rifampin)	Antituberculosis
Tacrine	Alzheimer's disease
Tolcapone	Parkinson's disease
Trovafloxacin	Antibiotic
Valproic acid	Anticonvulsant
Zafirlukast	Asthma
Zileutin	Asthma
Cholestatic injury	
Terbinafine	Antifungal

*Elicits allergic and non-allergic mechanisms. CNS, central nervous system.

more significance than increased ALT levels alone. It is therefore reasonable to apply the lower bilirubin elevation as a threshold for heightened concern, but probably not correct to apply the Hy's law 10% fatality (or liver transplantation) rule to predict the incidence of severe liver injury. This simply reflects the fact that a considerable amount of liver damage is required to raise serum bilirubin and, in general, serum bilirubin of 4 mg per dl indicates more injury than 2 mg per dl. Several recent examples of idio syncratic toxins met the FDA criteria for Hy's law in clinical trials, although acute liver failure (coagulopathy and encephalopathy) did not occur in the study population (but was observed post-marketing). Examples include troglitazone (discussed later), trovafloxacin and bromfenac.

Clinical signature

The clinical signature of drug-induced liver disease parallels all acute and chronic liver diseases. However, the bulk of adverse reactions are present as acute hepatocellular injury associated with increased serum transaminases, minimal serum alkaline phosphatase elevation and variable jaundice (resembling hepatitis); acute cholestatic injury associated with marked increased alkaline phosphatase, mild increased transaminases and jaundice; or a mixed pattern with combined features of hepatocellular and cholestatic injury (marked increased serum transaminases and alkaline phosphatase). The cholestatic pattern is usually not life-threatening but is often characterized by a more prolonged jaundice after drug withdrawal. Individual drugs tend to produce a signature in this spectrum that is characteristic for the drug, but exceptional cases do occur. For example, when troglitazone caused injury, it was usually hepatocellular[10,23], but on rare occasions the injury was mainly cholestatic[24]. Augmentin usually causes cholestatic injury but occasionally has been associated with acute liver failure[25].

Cholestatic injury results in some cases from inhibition of bilirubin or bile-salt transport (for example, cyclosporin and oestrogen metabolites)[26]; this is referred to as 'bland' CHOLESTASIS because the pathology shows no inflammation or necrosis. More commonly, cholestatic reactions seem to be associated with some degree of CHOLANGIOCYTE injury, which may or may not be accompanied by significant hepatocellular injury and inflammation. In some cases, excretion of a toxic metabolite into bile has been suggested (as, for example, in the case of terbinafine[27], flucloxacillin[28] and α-naphthylisothiocyanate[29]), which might then attack cholangiocytes. More commonly, however, idiosyncratic cholestatic liver injury seems to be allergic (TABLE 2), on the basis of injury features, such as rash, eosinophilia, rapid positive rechallenge (for example, Augmentin[25], erythromycins[30] and chlorpromazine[31]). It is of interest that a recent assessment of HLA status suggesting a genetic predisposition to allergy showed an association with cholestatic/mixed idiosyncratic hepatotoxicity[32].

Less commonly encountered pathological types of liver injury can occur with certain drugs. These include microvesicular STEATOSIS, non-alcoholic STEATOHEPATITIS, chronic hepatitis, CIRRHOSIS, veno-occlusive disease, PELIOSIS HEPATIS and benign and malignant neoplasia. Due to space limitations, these unusual reactions will not be discussed.

Lessons from troglitazone and beyond

The experience with troglitazone is very instructive, and several thousand patients have participated in clinical trials of the drug. The incidence of ALT greater than three times ULN in treated patients was 1.8%, versus 0.6% in placebo controls. ALT greater than ten times ULN was seen in 0.6% of troglitazone cases and none of the controls. Overt hepatocellular injury (jaundice) occurred in two troglitazone subjects, but there was no instance of acute liver failure[33]. Therefore, roughly 1 in 1,000 patients had elevated ALT and jaundice, fulfilling the criteria for Hy's law, which predicts acute liver failure in 1 in 10,000. The increased incidence of mild ALT abnormalities, although worthy of closer scrutiny,

CHOLESTASIS
Stoppage or suppression of bile flow.

CHOLANGIOCYTE
Bile-duct epithelial cell.

STEATOSIS
Accumulation of fat in the liver.

STEATOHEPATITIS
The presence of fat in liver cells accompanied by inflammation and fibrosis.

CIRRHOSIS
A type of chronic, progressive liver disease in which liver cells are replaced by scar tissue.

PELIOSIS HEPATIS
Blood-filled spaces in the liver due to injury to endothelial cells.

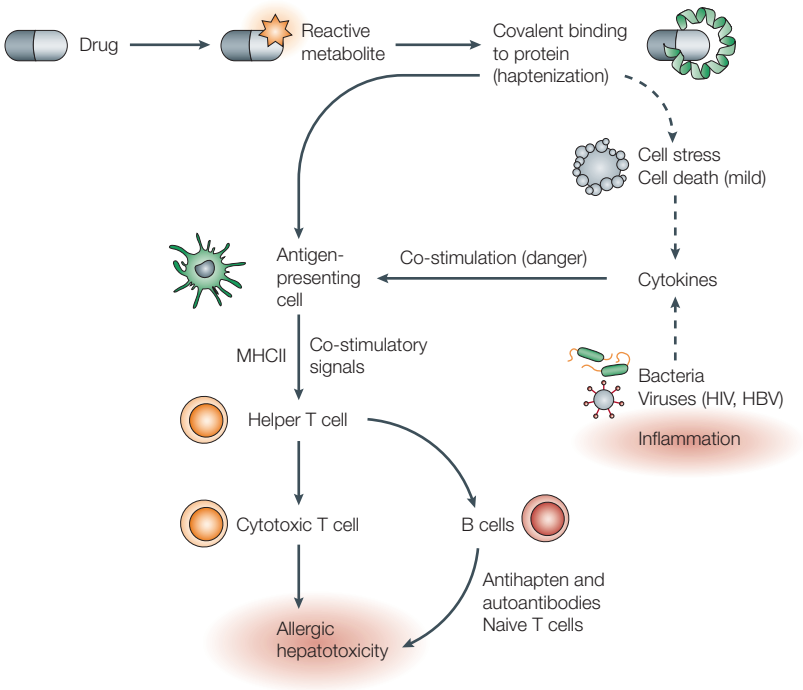

Figure 1 | **The danger hypothesis for immune-mediated idiosyncratic hepatotoxicity.** Hapten formation leading to major histocompatibility complex class II (MHCII) presentation of haptenized peptide by antigen-presenting cells (APCs) along with co-stimulation of APC signalling molecules by mild injury, inflammation or infection promotes helper T-cell activation leading to T-cell responses to the antigen. The cytotoxic T cells are then targeted against hepatocytes that express haptenized protein or MHCI presentation of haptenized peptides on the cell surface. Antibody to haptenized protein or concomitant autoantibodies could theoretically mediate and promote antibody-dependent cell-mediated hepatotoxicity.

was not necessarily predictive of the subsequent occurrence of more serious cases. Even the higher level of ALT abnormalities, although perhaps more concerning, cannot be viewed as a certain predictor of liver failure. For example, tacrine is associated with a very high incidence of marked ALT abnormalities, but jaundice is extremely rare[34,35]. However, the occurrence of troglitazone-associated overt hepatitis cases was a very serious predictor of liver failure. Indeed, in the post-marketing experience of about 2 million troglitazone-treated patients, nearly 100 cases of acute liver failure were reported to the FDA (1 in 20,000 patients taking the drug). Considering the known poor performance (only a small percentage) of the FDA's MedWatch system in capturing cases of toxicity in general, balanced against the fact that adjudication might eliminate as many as half the cases as being unlikely due to troglitazone, the predicted incidence of acute liver failure of ~1 in 10,000 is probably close to reality.

Recently, the thrombin inhibitor ximelagatran showed an even more significant signal in clinical trials, with an incidence of ALT greater than three times ULN in 7.8%, ALT greater than ten times ULN in 1.9%, elevated ALT with bilirubin greater than twofold in 0.5% of patients, respectively. Most importantly, several cases of acute liver failure occurred in the clinical trial population of nearly 7,000 patients[36]. This is a worse toxicity profile than most of the recently withdrawn or restricted idiosyncratic hepatotoxic drugs (TABLE 1), none of which were associated with acute liver failure pre-marketing. Certainly, there are several currently marketed drugs with toxicity profiles in this range (for example, isoniazid, pyrazinamide, bosentan and trovafloxacin[37,38]). The difficult question is when the risk–benefit analysis favours approval (perhaps restricted) or withdrawal. This is a complex issue and the decision is usually based on several factors: the disease (for example, in cancer the severity of the disease might justify risk of side-effects); acute versus chronic use of the drug (for example, a diabetes drug would need to be safe for long-term use); safety and efficacy of existing medication; and the likely efficacy of a risk-management plan (for example, restricted use and monitoring). There seem to be no hard-and-fast rules for making the final decision and each case has to judged on its own merits.

Role of ALT monitoring

A crucial issue is whether serum ALT monitoring during drug treatment can prevent the occurrence of life-threatening idiosyncratic hepatotoxicity by enabling the early recognition of injury and cessation of drug administration before serious problems occur. There seems to be little justification for this approach in the allergic group because these reactions occur early, usually progress very rapidly to become symptomatic and are therefore easily recognized. The non-allergic cases associated with delayed toxicity might be suitable for such a risk-management plan. However, there are a number of problems. First, in the absence of a simple, readily accessible screening tool for liver injury (either self-administered or given by a pharmacist), the most practical approach is monthly monitoring. More frequent monitoring has occasionally been recommended (for example, for tacrine and pemoline) but the decline in cases of hepatotoxicity associated with these drugs has arguably been due to reduced usage rather than the efficacy of monitoring[39]. Monthly monitoring was recommended for troglitazone, but a major problem was compliance. David Graham of the FDA reported that even after three warning letters were sent from the manufacturer of troglitazone to physicians recommending baseline and monthly ALT level monitoring (amid much publicity surrounding the drug), examination of a health-maintenance organization database revealed that of new patients placed on the drug, 45% had a baseline test and only 33% at 1 month and 13% at 5 months were tested[40]. Clearly, compliance with monitoring is very poor. However, it is conceivable that more rigorous risk-management programmes that limit monthly prescription refills according to the results of serum ALT monitoring might overcome this problem, but would entail considerable cost and probably inhibit use. Finally, there is the question of the actual efficacy of monitoring. The problem is that successful monitoring needs to identify cases before an irreversible process is set in motion. Graham reported that of 12 cases of acute liver failure from troglitazone in the FDA records in whom monthly monitoring was

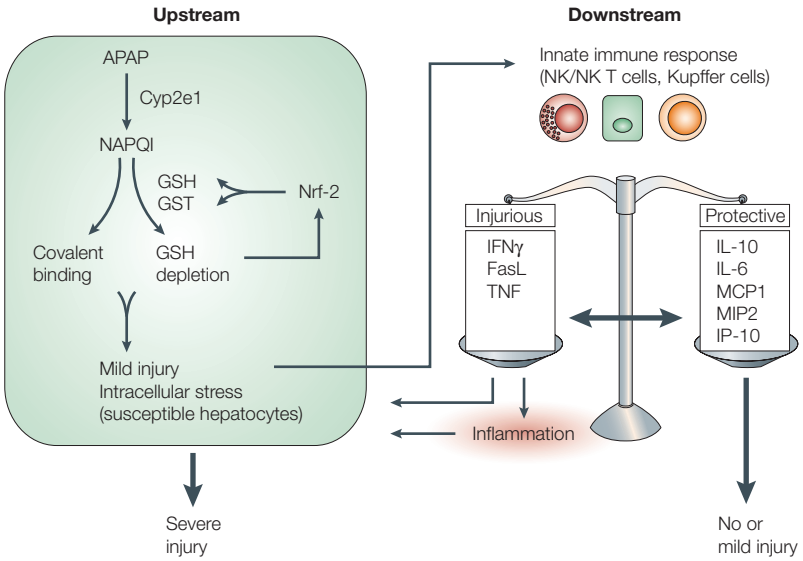

Figure 2 | **Current concepts of experimental acetaminophen (APAP) hepatotoxicity.** Upstream events in hepatocytes lead to exposure to NAPQI which undergoes covalent binding after preferential depletion of glutathione (GSH). A protective response mediated by the transcription factor NRF2 modulates the toxic threshold. Upstream events promote intracellular stress and mild injury activates the downstream innate immune system, which represents a balance of pro- and anti-inflammatory responses, the interplay of which determines progression to severe injury or no injury. APAP, acetaminophen; FasL, Fas ligand; GSH, glutathione; GST, GSH S-transferase; IFN, interferon; MCP1, monocyte chemoattractant protein 1; MIP2, macrophage inflammatory protein 2; NK, natural killer; TNF, tumour-necrosis factor.

actually performed, the liver injury progressed from normal ALT to acute liver failure within 1 month in 9 cases[10]. Therefore, waiting for an ALT level to exceed greater than three times ULN might be too late. Alternatively, lowering the ALT threshold for drug cessation might improve the efficacy somewhat but at the cost of greatly amplifying the number of withdrawn patients who would not have developed more severe toxicity. At present we are left with a conundrum where monitoring liver function is concerned: on the one hand we have poor compliance, unconvincing efficacy and far more patients withdrawn from treatment than would actually experience a serious problem. On the other hand, logic suggests that for diseases where the risk/benefit ratio favours continued availability, strict monitoring might have some benefit compared with no monitoring at all.

The experience of monitoring for injury during isoniazid treatment is also of interest. It has been argued that monthly interrogation for the occurrence of symptoms of liver injury is sufficient when isoniazid is used as chemoprophylaxis. The setting for isoniazid treatment is usually a public-health agency — that is, patients form a captive audience and undergo mandatory monthly visits for prescription refills. This approach was reported to be very effective in avoiding hospitalizations for hepatotoxicity without serum ALT monitoring[41]. However, the population studied was relatively young (<35 years of age), and it is recognized that isoniazid toxicity increases with age; therefore the efficacy of this approach in older patients is not convincing. Furthermore, the incidence of hepatotoxicity from antituberculosis medications increases greatly when they are used in combination[38,41], so it is unwise to extrapolate from the public-health chemoprophylaxis studies of young patients to older (>35 years of age) individuals or those being treated for active tuberculosis.

Predicting idiosyncratic hepatotoxicity

Remarkably, no animal models exist for allergic or delayed non-allergic idiosyncratic hepatotoxicity, or which reproduce the phenomenon of transient hepatotoxicity, which disappears with continued use (through physiological adaptation). Furthermore, most of the idiosyncratic hepatotoxins that have reached clinical trials or marketing showed little or no evidence of toxicity in animal toxicology studies. This is not to say that animal toxicology studies are worthless[42] — certainly, many chemicals that would have been dangerous to patients have been identified and development of the compound suspended. Even this is imperfect because some drugs that are toxic in certain species pose little risk to patients (for example, statins).

The pharmaceutical industry has been exploring applications of the new technologies of toxicogenomics, proteomics, metabonomics and so on to animal toxicology in an attempt to develop profiles or signatures of certain toxicities[43–46]. The approach has been to use panels of drugs and chemicals to identify patterns of changes in gene expression and so on at sub-toxic exposures of the drugs/chemicals that might be predictive of hepatotoxicity. Although this would seem a logical and potentially fruitful approach, it has not yet realized its potential, nor is it certain that it will. The biggest barrier is that we do not know exactly what we are looking for, either in terms of understanding the mechanisms of idiosyncratic hepatotoxicity and the role of universal mechanisms of cell toxicity, or to what extent the problem is specific to an individual drug or class. Similarly, testing drugs in cultured human hepatocytes has recently received interest. Problems with this approach include the unphysiological nature of the model and the high drug exposures needed to observe effects. For example, very high concentrations of troglitazone in the absence of albumin binding rapidly kill 100% of hepatocytes[47–49]. How does this relate to delayed toxicity at therapeutic doses in the small subset of patients who are susceptible?

Another approach has been to screen drugs in development for covalent binding or interaction with glutathione (GSH) using subcellular fractions or cells, because this is thought to identify potential toxic metabolites. However, these phenomena are not necessarily predictive of clinical problems. The hope is that these new approaches using animals and cells, as well as the identification of reactive metabolites, will provide clues concerning pathophysiology, which might be relevant to susceptible humans. The approach is certainly worthy of investigation and could provide industry with criteria by which to make decisions about the utilization of resources regarding which drug leads to follow up or which might be risky. Although

one could discard many more chemicals than would actually cause a problem in patients, it is reasonable to assume that some of the chemicals that exhibit these types of preclinical signatures will be problematic. However, the field of preclinical toxicology is likely to see the greatest progress when we know precisely what we are looking for. In that case, knowledge of the genetic and environmental contributors to human idiosyncratic reactions can be exploited to focus animal toxicology studies so that liver injury can be unmasked by using animal models with the relevant genetic variations that determine human susceptibility.

APAP: mechanistic for idiosyncratic toxicity

It is noteworthy that the bulk of our knowledge of hepatotoxicity derives from the experimental model of APAP hepatotoxicity. Certainly, APAP is one of the few hepatotoxic drugs that provide an experimental animal model. But is clinical APAP liver injury relevant to idiosyncratic hepatotoxicity and can we gain insights from this model that can be extrapolated to the mechanisms of idiosyncratic hepatotoxicity?

APAP is the leading cause of life-threatening acute hepatotoxicity in the US. More than one-third of the cases of acute liver failure in the US are due to APAP[1]. With few exceptions, this occurs when recommended doses of APAP are exceeded. It is widely recognized that a single massive suicidal ingestion leads to toxicity. However, it is estimated that about half of the cases of acute liver failure due to APAP are associated with unintentional or accidental ingestion of high doses in a more sub-acute fashion (days to weeks)[1]. This is not to say that suicidal single ingestions are not encountered far more commonly, but the prompt use of the antidote *N*-acetylcysteine limits the occurrence of liver failure. The reasons for unintentional overdosing are complex, and include lack of awareness, the presence of APAP in many different products that are used in combination and APAP–opiate abuse. It is likely that far more individuals consume excessive amounts of APAP than develop overt or life-threatening toxicity. Indeed, individuals have been described who consume massive daily quantities without a problem[50]. So it seems reasonable to suggest that APAP (especially in individuals who exceed recommended dose limits of 4 g per day and, perhaps in rare cases, in individuals who consume therapeutic doses) is an idiosyncratic hepatotoxin, notwithstanding its well-recognized dose-related effects.

Animal models of APAP toxicity support this hypothesis and provide mechanistic insights that might be applicable to human idiosyncratic hepatotoxicity. Susceptibility to APAP toxicity can clearly be increased or decreased in various circumstances. Indeed, examination of these circumstances, particularly genetic manipulations, could be very informative in providing clues to direct research efforts in clinical situations in which idiosyncratic hepatotoxicity has been identified.

APAP metabolism is well described[51]. The minor electrophilic cytochrome P450 (CYP) metabolite *N*-acetylbenzoquinoneimine (NAPQI) has a key role in

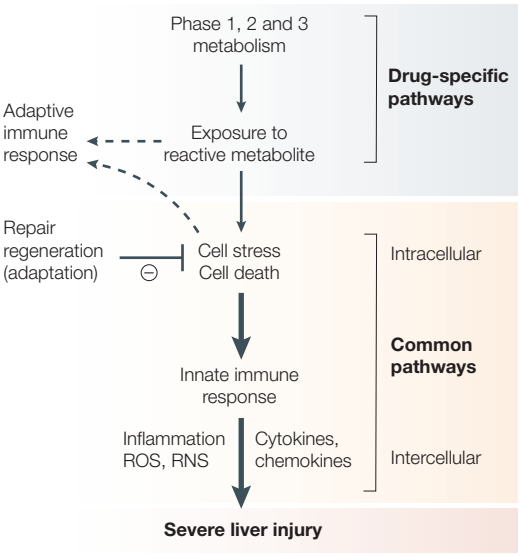

Figure 3 | **Drug-specific and common pathways of idiosyncratic drug hepatotoxicity.** Exposure to reactive metabolites depends on status of cytochrome P450 proteins (CYP450; phase 1), conjugations (phase 2) and transport (phase 3). These are specifically related to the nature of the chemical entity. However, exposure to the reactive intermediate is a prerequisite that is necessary but not sufficient for toxicity. Upon exposure to the reactive metabolite it is speculated that common pathways of cellular and organ injury are called into play. Therefore genetic and environmental influences on the determinants of exposure to the specific reactive metabolite or the more common pathways of response to the metabolite can contribute to liver injury. RNS, reactive nitrogen species; NOS, reactive oxygen species.

APAP metabolism, and GSH preferentially detoxifies the metabolite. Toxicity occurs when nearly all the GSH (including mitochondrial) is consumed, which means depletion of GSH at a rate that exceeds its replenishment. Consequently, factors that affect NAPQI production and detoxification influence susceptibility to APAP toxicity. For example, Cyp2e1-null mice are protected from toxicity[52], whereas induction of Cyp2e1 increases susceptibility[53]; doubling Cyp2e1 halves the threshold for a toxic dose[54,55]. GSH synthetic and detoxification enzymes are regulated by the transcription factor NRF2[56]. *Nrf2*$^{-/-}$ mice are more susceptible to APAP toxicity[57]. Although this is no surprise, it is of interest that Nrf2 in wild-type mice is activated by even subtoxic doses of APAP[56], indicating that the threshold for toxicity is modulated by the rapid activation of this transcription factor. Certainly, in extrapolating these findings to idiosyncratic hepatotoxicity from various drugs, genetic predisposition due to polymorphisms that affect basal expression of genes that control drug metabolism and detoxification, as well as the transcription factors that respond to initial stress to modulate rapid changes in the expression of target genes, make sense as upstream candidates (FIG. 2).

There have been a number of investigations in recent years of downstream factors that come into play once NAPQI is formed and GSH has been consumed,

and the innate immune system has emerged as an important modulator of the progression and severity of organ damage. Knockout or mutant mice that lack either interferon-γ (IFNγ), Fas or Fas ligand (FasL) are resistant to APAP toxicity[58,59], whereas knockout of interleukin-10 (IL-10) and IL-6[60,61] is associated with increased susceptibility to toxicity. Indeed, mice lacking IL-10 experience APAP toxicity at doses that are not toxic in wild-type mice and are only several-fold greater than the maximum recommended for humans. In most of these knockout models, protection or worsening was not associated with alterations of time course or extent of covalent binding or GSH depletion/repletion. These cytokine-knockout models demonstrate a key point, namely that in response to non-toxic doses of APAP there is stimulation of the expression of both pro- and anti-inflammatory cytokines. When the anti-inflammatory cytokine/chemokine response is diminished, the threshold for injury is lowered (and vice versa) (FIG. 3).

Natural killer/NK T (NK/NKT) cells seem to be crucial in the innate immune system's participation in hepatotoxicity of APAP[59]. Depletion of these cells removes the main source of IFNγ and therefore the subsequent pro-inflammatory cascade mediated by cytokines and chemokine production from NK/NKT cells themselves, as well as their targets (Kupffer cells, endothelial cells, stellate cells and hepatocytes). In addition, NKT cells can be directly cytotoxic. The signals that activate and recruit NK/NKT cells to the liver in the early stages of APAP hepatotoxicity have not yet been identified but could include cytokines produced by Kupffer cells (for example, IL-12) in response to initial hepatocyte stress or death. The prevention of NK/NKT-cell response (monoclonal antibody depletion)[59] or the absence of its major product, IFNγ (knockout)[58,59], markedly blunts the progression and severity of the subsequent APAP liver injury.

Models that modulate the innate immune response to APAP underscore three very important points. First, the occurrence and extent of organ injury are heavily influenced by events controlled by the innate immune system downstream of initial drug metabolism. Upstream metabolism/detoxification can therefore occur in a nearly identical pattern, with or without liver toxicity developing. As a result, covalent binding and GSH depletion do not necessarily kill the hepatocytes. Second, in response to unidentified signals (presumed to be related to stress of the upstream events), an interplay of cytokines and chemokines is activated that have counteracting effects leading to a tenuous balance. This is somewhat analogous to the danger model of adaptive sensitization, in which the innate immune system is also crucial. Circumstances that perturb the protection/injury balance of the innate immune factors (that is, various gene knockouts) cause the scales to tip in one direction or the other — that is, little or no injury or more severe injury. Third, the genetic perturbations of drug metabolism and detoxification (upstream) and the innate immune system (downstream), when applied to a 'predictable' APAP toxicity model, generate a circumstance similar to idiosyncratic human hepatotoxicity. A partial listing of these and other biological manipulations that have been reported in the APAP model is provided in TABLE 4. Genetic polymorphisms that influence the expression of cytokines, chemokines and so on therefore represent potentially fruitful targets for investigation in the clinical setting. Additionally, as injury occurs — particularly when gradual or sustained over days — the liver responds to cell death by activating the process of regeneration. Therefore another area worthy of exploration in the pathogenesis of idiosyncratic toxicity is the influence of genetics and environment on the regenerative process.

An important point emerges in considering the interplay of drug-induced hepatocellular stress/mild injury and the innate immune response. It is unlikely that the innate immune response would kill normal hepatocytes, so it is likely that the stress of toxic metabolites, GSH depletion or oxidative stress could render liver cells more susceptible to being killed by the innate immune response or the inflammation that is elicited by the innate response (FIGS 2,3). For example, normal hepatocytes are resistant to killing by tumour-necrosis factor (TNF), whereas acute GSH depletion[62], as well as inhibition of protein or RNA synthesis, make the hepatocytes susceptible. A major factor in these situations is the interference with nuclear factor-κB (NF-κB) activation or trans-activation and survival gene expression in response to TNF-induced NF-κB activation. Although the role of TNF in APAP toxicity is controversial, APAP does sensitize cultured hepatocytes to TNF-induced apoptosis when antioxidants are used to suppress oxidative stress-induced necrosis[63].

Mode of cell death

The relative contribution and importance of apoptosis versus necrosis is an open question in idiosyncratic drug hepatotoxicity. The distinction between the two modes of cell death is probably of more than just theoretical interest. Apoptosis might be less injurious, especially when not massive, because of rapid removal, whereas necrosis might be more likely to promote inflammation and consequent collateral damage[64]. Even this distinction is not absolute, because apoptosis can be pro-inflammatory and death ligands, such as FasL, can induce inflammation even in the absence of apoptosis[65,66]. Immune-mediated killing of hepatocytes is mainly achieved by cytotoxic T cells through an apoptotic mechanism mediated by FasL and granzyme B/porin[67,68]. The role of antibody-dependent cell-mediated cytotoxicity is less certain. *In vitro* demonstrations of the latter phenomenon with antisera from patients with halothane[69] and tienilic acid toxicity[70] are of interest but do not prove that this mechanism is important *in vivo*. In non-allergic idiosyncratic hepatotoxicity, the role of apoptosis is largely unknown. Although some drugs, such as troglitazone, induce apoptosis of cultured hepatocytes, it is not clear whether this observation has *in vivo* relevance. However, in theory apoptosis can be triggered by certain idiosyncratic hepatotoxins and is a mechanism of idiosyncrasy well worth considering because it might

Table 4 | **Susceptibility to APAP toxicity in experimental models**

Treatment/model	References
Decreased susceptibility	
CAR-null mice	77
GST-pi-null mice	78
CYP2E1-null mice	52
Interferon-γ-null mice and anti-IFNγ	58,59
FasL-deficient (gld) mice	59
Fas-deficient (lpc and antisense) mice	59,79
IP-10 and MIP-2 administration	80,81
IL-6 administration	61
IL-11 administration	82
Natural killer (NK) and NK T-cell depletion	59
LPS-binding-protein-null mice	83
Increased susceptibility	
GSH depletion	84
Induction of Cyp2e1	53
NRF2-null mice	57
IL-10-null mice	60
IL-6-null mice	61
COX2-null mice	85
Kupffer cell depletion	86
CCR2-null mice	87
Anti-MIP2 antibody	88
Agonistic anti-Fas pretreatment	89

*The roles of tumour-necrosis factor and nitric oxide are controversial: some studies indicate either a protective or aggravating role. Adapted from REF. 90. CAR, coxsackie and adenovirus receptor; CCR2, chemokine receptor 2; COX2, cyclooxygenase 2; CYP2E1, cytochrome P450 2E1; gld, generalized lymphoproliferative disease; GSH, glutathione; GST, glutathione *S*-transferase; IL, interleukin; IP-10, interferon-γ-inducible protein 10; lpr, lymphoproliferation; MIP2, macrophage inflammatory protein 2; NRF2, nuclear factor E2-related factor 2.

involve the specific action of drugs and metabolites in the apoptosis cascade, whereas necrosis might be more likened to an overwhelming bioenergetic catastrophe. For example, a drug could directly affect mitochondria or a specific step in the apoptotic cascade, or could, by contrast, indirectly promote apoptosis-inducing intracellular stress (for example, by causing oxidative stress, endoplasmic reticulum or cytoskeletal stress, or DNA damage[68]) leading to GSH depletion, inhibitory effects on protein or RNA synthesis and diminished proteosomal degradation, which might sensitize hepatocytes to death-receptor-mediated apoptosis. The choice of apoptosis versus necrosis can be determined by a number of factors, including ATP status. So conditions in which a more severe hit to mitochondria occurs might favour necrosis (for example, APAP), whereas a less severe hit to mitochondria or anaerobic glycolytic production of ATP to sustain ATP above a critical threshold could favour apoptosis[71]. It is intriguing to speculate that the choice between apoptotic or necrotic cell death, even if the total magnitude of cell death is no different, could influence the severity of organ damage, perhaps by affecting the extent of innate immune response and inflammation. Genetic variations in the levels and response of pro- versus anti-apoptotic genes therefore should not be ignored in future investigations of the risk factors for idiosyncratic hepatotoxicity.

Risk factors

There are several crucial questions to consider in evaluating the factors that determine susceptibility to uncommon idiosyncratic reactions. First, are there drug-specific factors either related to the chemical properties of the drug and metabolite; the unique handling of the drug by the various components of phase 1, 2, or 3 of drug metabolism (variation in handling between different phases of drug metabolism); or the pharmacodynamic effects of the particular class of drugs? It is clear that genetic polymorphisms, or the effects of concomitant drugs, alcohol, nutrition or disease, can alter the threshold for exposure to toxic metabolites. Drug-class-related adverse effects on the liver have rarely been observed; statins represent one of the few clearcut examples. On the other hand, cross-sensitization to the immuno-allergic response can be seen within certain classes of drugs, such as halogenated anaesthetics, phenothiazines, erythromycins, tricyclics and anticonvulsants.

Second, are there factors that are unrelated to the type of drug but instead represent the downstream response (converging common pathways) to the initiating events — for example, cell-death cascades, innate immune response, repair or adaptation or liver regeneration? (FIG. 3). These biological processes have inherent variability and can be influenced by genetic and environmental factors.

Third, are the risk factors that determine the susceptibility to more frequent mild injury of any importance in the susceptibility to the rare, more severe, symptomatic idiosyncratic hepatotoxicity? It seems likely that the occurrence of rare reactions depends on multiple factors: a reasonable hypothesis (unproved) is that the determinants of mild injury are prerequisites for the more severe reactions, the latter requiring the contribution of one or more additional rare determinants. This concept accords with the hypothesis (again unproved) that the low-grade and more frequent injury reflects genetic polymorphisms in phase 1–3 of upstream hepatic drug handling or intracellular detoxification, but that this mild injury becomes more severe if environmental factors (for example, viral disease), or genetic polymorphisms or rare mutations, modulate how the innate immune system or other downstream biological processes respond to the upstream stress at that particular point in time and space. Only a small number of genetic susceptibility studies have been published in this field, most of which have assessed genetic determinants of the mild cases with asymptomatic ALT elevations in clinical trials. Both troglitazone- and tacrine-induced mild injury has been associated with GSH *M1* and *T1*[72,73], tolcapone with *UGT1A6*[74], and isoniazid with

N-acetyl transferase (*NAT2*) and *CYP2E1* polymorphisms[75]. These are all upstream factors that can contribute to, but do not fully account for, susceptibility and are of uncertain significance in influencing the occurrence of severe hepatotoxicity.

Furthermore, conflicting results have been reported with isoniazid and tacrine. Interestingly, one small study found an association between diclofenac toxicity (a mix of mild and severe cases) and polymorphisms of IL-10 and IL-4[76]. Single nucleotide polymorphism (SNP) analysis and sequencing of the entire genome in population studies, such as clinical trials, is now technically and economically feasible and will hopefully shed more light on the problem of idiosyncratic hepatotoxicity. Furthermore, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Drug Induced Liver Injury Network (DILIN) project comprises a group of university medical centres that has cast a wide net to collect bona fide idiosyncratic hepatotoxicity cases with a view to refining causality assessment criteria by focusing on a group of drugs recognized as idiosyncratic hepatotoxins. Information on a sufficient number of severe cases should enable a thorough examination of the biological downstream genetic determinants of hepatotoxicity

Various environmental risk factors have been suggested as contributors to idiosyncratic hepatotoxicity. Underlying all of these risks is the usual preponderance of females in both allergic and non-allergic cases. A wide variety of other factors have been noted, such as age (youth for valproic acid and old age for isoniazid), obesity (halothane), possibly nutritional deprivation (APAP), chronic hepatitis B and C (isoniazid), HIV (sulphonamide hypersensitivity and isoniazid), possibly alcohol abuse (APAP, isoniazid, methotrexate) and drug interactions and effects of concomitant medications (phenobarbital and valproic acid, combination of antituberculosis drugs).

Conclusions

Idiosyncratic hepatotoxicity associated with jaundice and symptoms occurs in a very small proportion of exposed individuals, mostly ranging from 1 in 500 to 1 in 50,000. Lower incidences are difficult to distinguish from the background incidence of idiopathic hepatitis (~1 in 100,000 adults annually) or acute liver failure of unknown aetiology (~1-2 in 1,000,000). The incidence of idiosyncratic reactions is too low to be simply accounted for by polymorphism of a single gene (by definition >1 in 100). Therefore, the interplay of multiple genetic and environmental factors must converge to determine these rare adverse events.

The challenge we face is in identifying these factors so as to avoid the use of problematic drugs in the rare susceptible individual, which would obviate the need for ALT monitoring, and perhaps even allow the safe use of the drug in the subset who develop the mild, transient injury. Major efforts in the pharmaceutical industry are being directed at defining chemical structures, reactive metabolites, oxidative stress and toxicogenomic/biological signatures in animals and cells that are potential characteristics of hepatic toxicity to guide drug development in an efficient, cost-effective manner. However, real progress in this field is likely to only come when the specific (drug metabolism) and more general or common pathway (cell death, innate immune response, repair and regeneration) determinants of both common mild and rare severe idiosyncratic hepatotoxicity are identified. Aside from providing an understanding of the mechanisms of toxicity, this approach will offer opportunities to design new strategies of preclinical toxicology using genetically modified animals and cells to unmask the toxic potential of chemicals that are being screened for clinical evaluation of safety and efficacy.

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Competing interests statement

The author declares competing financial interests: see Web version for details.

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