

The roles of drug metabolism in the pathogenesis of T-cell-mediated drug hypersensitivity

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Purpose of review

The purpose of this review is to highlight recent studies on drug metabolism in T-cell-mediated reactions. Although the hapten theory and the danger hypothesis imply an important role of reactive metabolites in the pathogenesis of drug hypersensitivity, the more recent pi concept gives the central role to the parent drug. It is therefore important to have a broad vision on data supporting each theory to understand the potential role(s) of drug metabolism in T-cell-mediated hypersensitivity.

Recent findings

Reactive metabolites have been identified for most drugs associated with hypersensitivity. Recent studies have further characterized drug metabolism outside the liver, in key tissues such as the skin and immune cells. Localized drug metabolism is associated with oxidative stress, adduct formation and toxicity creating danger signals for antigen presenting cells, influencing whether a drug antigen will induce tolerance or immunity.

Summary

The involvement of metabolic drug activation in the initiation and propagation of hypersensitivity is intriguing since metabolites are generated in different quantities throughout the body, can be directly or indirectly toxic to cells, might stimulate innate immunity, and can bind to protein to generate neoantigens for cellular and humoral responses.

Keywords

cytotoxicity, danger signal, drug metabolites, drug metabolizing enzymes, drug–protein adducts, hapten theory, oxidative stress, pharmacogenomics, pi concept, T cells

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Introduction

Adverse drug reactions (ADRs) remain an important cause of hospitalizations, and are associated with fatal cases every year [1,2]. Some ADRs appear dose-independent, without any relationship with the pharmacological effect of the drug, and are therefore unpredictable. When immune-mediated, such idiosyncratic events are referred to as hypersensitivity reactions. Despite decades of investigation, their exact pathogenesis remains unknown.

Three theories, however, coexist to try to explain molecular events leading from a therapeutic drug exposure to an immune reaction. All three are based on multiple pieces of clinical, epidemiological, and experimental evidence (Table 1). Firstly, the hapten or prohaptent hypothesis has been the centre of investigation to understand the pathogenesis of immune-mediated ADRs. This theory is based on the fact that drugs are thought to be too small to stimulate an immune response. They would therefore need to bind to a protein (hapten hypothesis),

or to be metabolized to a protein reactive metabolite (prohapten hypothesis) to sensitize the immune system [3,4]. In addition, the danger theory is based on the fact that the immune system requires more than an antigen to trigger a full immune reaction. ‘Danger signals’, such as the expression of costimulatory molecules by antigen presenting cells, oxidative stress, or cell death, would lead the immune system towards an active immune response rather than tolerance [5,6]. Finally, a new theory has emerged from studying drug-specific T-cell clones in a few drug hypersensitivity reactions. Some drugs seem to be capable of directly interacting with immune receptors [major histocompatibility complex (MHC) and T-cell receptor (TCR)] to activate T cells. This phenomenon appears similar to a normal interaction between a drug and its pharmacological receptor target, and was therefore named ‘the pharmacological interaction with immune receptors’, or pi concept [7,8].

The present review will give an overview of the potential involvement of drug metabolites, direct or indirect, in the

Table 1 Evidence for the different theories of drug allergy

(Pro) hapten theory	pi concept	Danger hypothesis
In-vivo drug–protein adducts	NDA	NDA
Ex-vivo adducts	NDA	Some drug–protein adducts cytotoxic
Known reactive metabolites	Absence of known reactive metabolites for some drugs associated with hypersensitivity reactions	Metabolite cytotoxicity
Activation of innate immunity by reactive metabolites	Activation of innate immunity by parent drug	Activation of innate immunity by reactive metabolites
Absence of any episode of clinical signs seen during drug hypersensitivity reaction, before drug exposure	Drug hypersensitivity reactions in parallel of virus reactivation	Drug hypersensitivity reactions in parallel of virus reactivation
Antioxidant deficiencies in HIV patients	Higher incidence of drug hypersensitivity in autoimmune diseases and viral infections	Higher incidence of drug hypersensitivity in autoimmune diseases and viral infections
Metabolite-specific T cells	Very low frequency of metabolite-specific T clones	NDA
Delayed onset of most T-cell-mediated drug hypersensitivity	Fast onset of some T-cell-mediated skin drug allergies	NDA
Antidrug antibodies	NDA	NDA
Antitissue antibodies as markers of humoral immunity	NDA	NDA
Positive patch tests (skin drug bioactivation)	Positive patch test (if absence of skin bioactivation)	NDA
Requirement of APC for PBMC drug-specific proliferation and cloning	Negative pulsing assays	Requirement of APC for PBMC drug-specific proliferation and cloning
Antioxidant deficiencies in HIV patients	NDA	Antioxidant deficiencies in HIV patients
Kinetics of T-cell activation compatible with processing and presentation of an drug–protein adduct formed by APC itself	Fast kinetics of T-cell clones or hybridoma activation	NDA
Metabolite, but not parent drug, immunogenic	NDA	Parent drug can be immunogenic when administered with FCA
Correlation between drug metabolizing enzyme polymorphisms and drug hypersensitivity	Correlation between certain HLA polymorphism and drug hypersensitivity	Correlation between TNF α promoter polymorphism and carbamazepine hypersensitivity

APC, antigen presenting cells; FCA, Freund's complement adjuvant; HLA, human leukocyte antigen; NDA, no data available; PBMC, peripheral blood mononuclear cells; TNF α , tumor necrosis factor alpha.

pathogenesis of T-cell-mediated drug hypersensitivity reactions.

Drug metabolites

Even though the main purpose of drug metabolism is to protect the body against drug toxicity, toxic metabolites have been identified for many drugs associated with drug allergy (Fig. 1), including hydroxylamine and nitroso sulfamethoxazole (SMX) for SMX [9], glycineylidide and monoethylglycineylidide for lidocaine [10], iminoquinone and arene oxide metabolites for carbamazepine [11,12], N-chloro, N-oxide and arene oxide metabolites for lamotrigine [13,14].

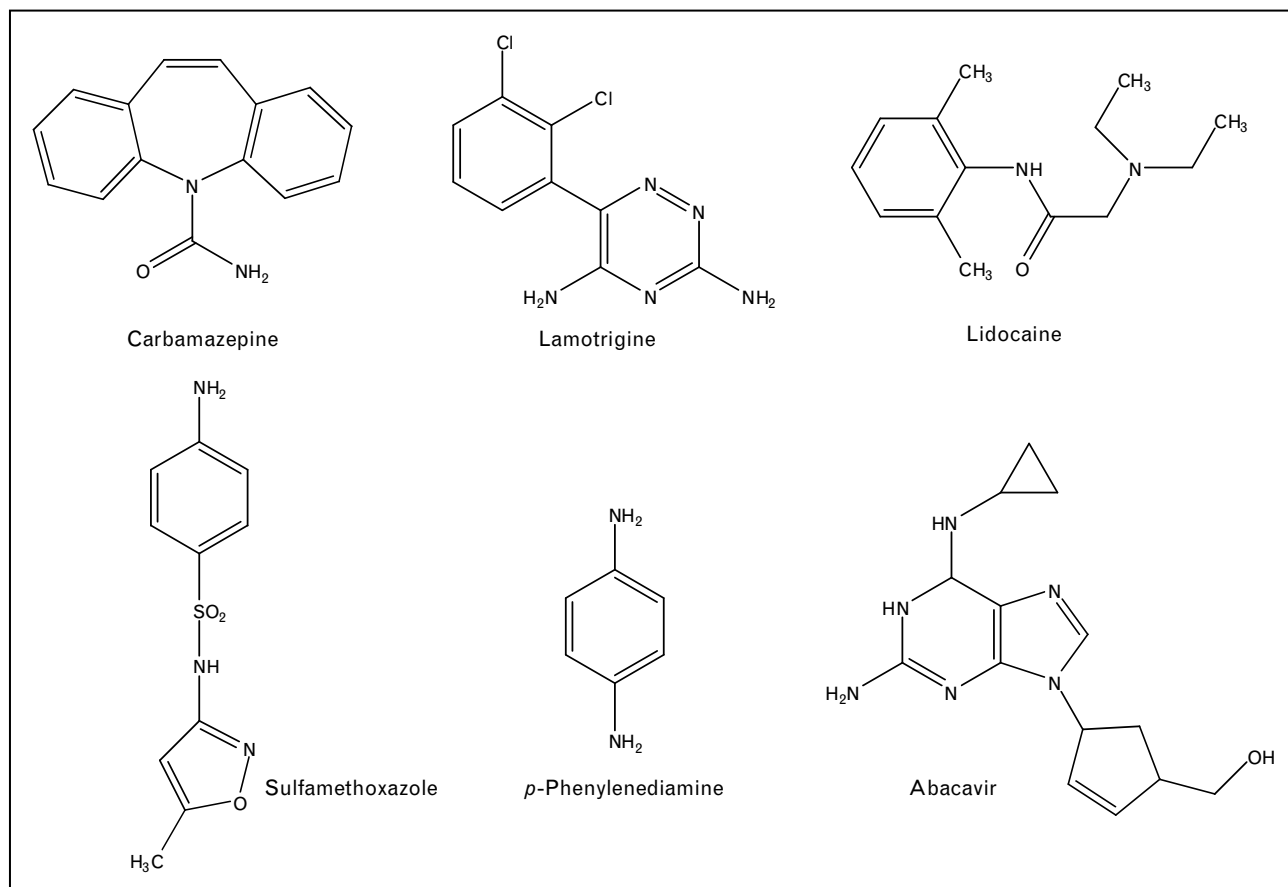
It is important to note that the absence of detectable reactive metabolites does not necessarily mean that a drug is not bioactivated, as this metabolite could be too reactive to be detected with techniques available. Furthermore, a reactive metabolite could be formed in insufficient quantities to reach detection limits of bio-analytical methods. Interestingly, some researchers have shown that peptide loaded MHC molecules could serially trigger several TCRs [15]. Others [16–18] proved that

T cells require as little as a few hundred receptor ligand interactions for activation. It is therefore possible that reactive metabolites, generated locally in quantities too low to be detected, could still be involved in an immune response.

Drug metabolism

Most of drug metabolism occurs in the liver. However, liver-generated metabolites are unlikely to stimulate T-cell-mediated reactions in other organs (cf. skin), unless the metabolite avoids detoxification processes, and is stable enough to circulate [19,20]. Although the liver is the main site of drug metabolism, it is known that other organs express drug-metabolizing enzymes, such as the skin or immune cells themselves.

For the past decade, more emphasis has been put on characterizing the drug metabolizing potential of the skin and its potential role in drug allergy. It has been shown by several groups that several cell types of the skin (keratinocytes, fibroblasts, Langerhans cells; and melanocytes) express both phase I and II enzymes, as well as transporters [21,22*,23]. Skin cells express less

Figure 1 Drugs and chemicals associated with delayed hypersensitivity reactions

Carbamazepine, lidocaine, lamotrigine, sulfamethoxazole, and abacavir are commonly studied for their association with T-cell-mediated allergies to drugs, while PPD is used as a model of chemical-induced T-cell-mediated reactions. All of these compounds have well characterized reactive metabolites, which could potentially play a role in the pathogenesis of such immune-mediated reactions. PPD, *para*-phenylenediamine.

drug metabolizing enzymes than hepatocytes; however, skin is larger in terms of weight and surface area than the liver, and is also highly vascularized. Therefore, the skin could be as important as the liver in bioactivating certain drugs, and could play a specific key role in drug-induced skin reactions. Some laboratories have therefore tried to develop some skin CYP450 cocktails to better evaluate the role of drug bioactivation in skin drug allergy [24]. The role of skin metabolism has also been intensively studied in some specific drug-induced T-cell reactions such as carbamazepine, dapsone, SMX, and phenytoin [25,26,27*,28,29]. Finally, the fact that the skin can bioactivate drugs, also implies that a positive patch test to nonreactive drugs does not exclude a role for drug metabolites in T-cell-mediated drug allergy, as the drug could be bioactivated in the skin itself.

Immune cells themselves, granulocytes, lymphocytes, and antigen presenting cells, express multiple drug metabolizing enzymes [30*,31,32]. Reactive drug metabolites

formed inside immune cells could have various consequences; direct activation, cell death leading to secondary activation, processing and presentation without uptake in the case of antigen presenting cells. More data are also becoming available on drug metabolism in dendritic cells, which could play an important role in directing the immune system towards a reaction rather than tolerance (see paragraph below on innate immunity).

Drug metabolites and drug-protein adducts

The ability of some metabolites, or sometimes the parent drug itself, to form protein adducts has been mainly investigated *in vitro*. In drug-induced immune-mediated liver diseases, such drug-protein adduct formation has been correlated with the development of hepatic injury [33]. Drug-protein adducts have also been characterized *in vivo* in patients with T-cell-mediated drug allergy. For instance, SMX-serum protein adducts have been found at a higher frequency in hypersensitive individuals when

compared with tolerant controls, in both humans and pet dogs [34–36].

The exact nature of the antigen that triggers immune sensitization in drug hypersensitivity reactions, and the antigen(s) that subsequently stimulate the immune events leading to the clinical signs are not fully defined. The (pro)haptens hypothesis, stipulates that a drug–protein adduct is the antigen in both cases. To support this hypothesis, several authors were able to immunize animals by injecting drug–protein adducts. For instance, Worral and Dickinson [37] triggered a humoral immune reaction against rat serum albumin modified with a protein reactive metabolite of diflunisal, a nonsteroidal anti-inflammatory drug associated with hypersensitivity reactions in humans. More recently, Cheng *et al.* [38•] were able to induce a metabolite-specific T-cell response by dosing mice with mouse serum albumin modified by nitroso-SMX.

It is important to note that adduct formation *per se* is not always associated with pathogenic clinical manifestations. For example, drug–protein adducts have been found in humans and animals exposed to drugs and drug metabolites without the development of any adverse event [38•,39].

Furthermore, it is possible that drug–protein adducts could have a protective role [40]. When a reactive metabolite covalently binds to a protein, it is no longer available to damage other proteins, or to decrease the antioxidant pool. In this respect, several authors have found that there is no direct correlation between the amount of SMX–protein adducts and cell death [41,42]. Laverne *et al.* [43] also demonstrated that an increase in SMX–protein adducts could be associated with a decrease in in-vitro cell death.

Drug metabolites and cytotoxicity

Cytotoxicity associated with drug metabolites has been intensively investigated for SMX metabolites [44,45]. However, metabolite-induced toxicity has also been illustrated with most other drugs associated with drug hypersensitivity, such as carbamazepine [46], amodiaquine [47], clozapine [48], clindamycin [49], or diclofenac [50]. It is noteworthy that certain parent compounds associated with drug hypersensitivity have been shown to be cytotoxic, but the role of reactive intermediates in this toxicity has not been investigated. This is the case for two drugs associated with direct interactions with immune receptors; lidocaine [51,52] and celecoxib [53,54].

Several research groups have attempted to use cytotoxicity as a predictive and diagnostic tool, using either the

parent drug with or without a metabolizing system, or the drug metabolite itself when available [55–57]. However, results were not always convincing [58], probably because these assays were based on a direct dose-dependent drug toxicity on one given cell type, while drug hypersensitivity are dose-independent stimulation of the immune system in an undefined location (either directly according to the pi concept, or through drug–protein adducts in the prohaptens hypothesis). However, drug metabolite-induced cell death is thought to play an important role in drug allergy because it could eventually serve as a danger signal in the initiation of a drug-specific immune response [59].

If parent drug-mediated or metabolite-mediated toxicity is often well defined, the exact mechanism of such toxicity often remains unknown. A drug or its metabolite could be toxic through various mechanisms, such as oxidative stress. Carbamazepine, for instance, was shown to be associated with oxidative stress *in vivo* [60]. Dapsone-hydroxylamine is more cytotoxic against human keratinocytes than SMX-hydroxylamine, which correlates with the fact that the dapsone metabolite generates more reactive oxygen species than the sulfonamide metabolite [61]. Similarly, it was shown that nitroso-SMX-induced cytotoxicity is, at least partially, secondary to redox cycling between the nitroso and the hydroxylamine metabolites [43].

In addition, such drug-induced oxidative stress could impact on the immune balance without killing the cell. Indeed, redox imbalance has been shown to induce dendritic cell maturation (see section below on innate immunity) [62,63], and to influence the cytokine profile of T-cell clones [64]. Moreover, oxidative damage on proteins has been associated with the formation of autoantibodies in several autoimmune diseases, such as lupus and rheumatoid arthritis [65]. Even though oxidative stress caused by drug metabolism could therefore be involved in a drug-induced autoimmune reaction, this area remains largely unexplored.

On the other hand, the formation of a metabolite–protein adducts could be cytotoxic by altering an important protein function. In this instance, SMX-hydroxylamine was also shown to induce peripheral blood mononuclear cell (PBMC) death by inhibition of intracellular esterase activity [66]. Similarly, diclofenac metabolite-induced hepatotoxicity is thought to be due to mitochondrial function impairment [50].

Drug metabolites and innate immunity

Antigen presenting cells play a pivotal role in the induction of immune reactions. More specifically, dendritic cells are tissue immunological sentinels, which can

influence the immune system towards either tolerance or reactive immunity [67,68]. This makes dendritic cells a potentially key player in the pathogenesis of drug-induced immune reactions.

It appears that contact sensitizers, such as *para*-phenylenediamine (PPD) and dinitrochlorobenzene (DNCB), can trigger both maturation and activation of dendritic cells [69,70,71]. However, data on similar interactions between systemic drugs and dendritic cells are limited. Nonetheless, the little information available shows that the interaction between dendritic cells and reactive drug metabolites could take part in the events leading to a drug-induced immune reaction. Betalactams are directly protein reactive, they represent an interesting model; however, to study the role of drug-protein adducts since they do not require a preliminary step of bioactivation. Interestingly, Rodriguez-Pena *et al.* [72] showed that amoxicillin can induce partial maturation and activation of dendritic cells, and increases their capacity to stimulate drug-specific T cells in hypersensitive patients. It appears therefore possible that a hapten can act as both a maturation stimulus and an antigen in a drug-induced immune reaction. Dendritic cells exposure to SMX increases expression of the costimulatory receptor CD40, an important costimulatory molecule in drug immunogenicity, apparently through metabolic activation and generation of intracellular adducts [30]. Furthermore, in human antigen presenting cells (PBMC, lymphocyte T-depleted PBMC, monocyte-derived dendritic cells), abacavir causes secretion of the inflammatory cytokines TNF α and IFN γ [73,74], an increase in expression of the costimulatory molecule CD40, and a redistribution of heat shock protein 70 (HSP70) considered to be a 'danger signal' [74].

Drug metabolites and T-cell immunity

The lymphocyte transformation test has been used to detect drug-specific and metabolite-specific T cells [75]. In human patients with a history of a drug hypersensitivity, T cells reactive to the parent drug, or to its metabolite when available, can be identified [76–79]. Interestingly, in animal models of drug immunogenicity, after immunization with the reactive metabolite, animal T cells only recognize the metabolite, not the parent drug [80,81]. Since clinical signs of drug hypersensitivity are only seen in human patients, and not in animal models, it is possible to conclude that metabolite-specific T cells are only markers of drug immunogenicity, while T cells specific for the parent drug could be involved in clinical manifestations of the reaction. However, it is important to note that in-vitro assays exploring drug metabolite-specific T-cell responses provide the metabolite antigen directly in solution where potentially stimulatory concentrations can be associated with toxicity. Furthermore,

adducts are formed with abundant extracellular, such as serum albumin, and cell surface protein. Due to methodological difficulties, studies using mixed cell systems where adducts might be generated intracellularly in antigen presenting cells or target cells (cf. keratinocytes), have not been possible to date. As such, experimental data to validate the hapten hypothesis remain elusive.

T-cell cloning is a powerful experimental tool developed to study T-cell-mediated immune reactions. Even though metabolite-specific T-cell clones can be less frequent than parent-specific clones, they are detected in every patient tested [76,82,83]. The pi concept was based on experiments where T-cell clones were able to recognize the parent drug apparently in the absence of any drug metabolism and processing [84–86]. The pi concept applies principles of basic pharmacology to an immune-mediated event. It surmises that the sum of reversible binding interactions between a drug and an immune receptor is sufficiently strong to stimulate-specific T cells, such as seen with the metal ion nickel which can form coordinate bonds with both MHC and TCR [87]. If the parent drug can interact with immune receptors, it is possible that metabolites will retain some kind of affinity for these receptors too. In this respect, Schnyder *et al.* [78] demonstrated that nitroso-SMX can bind irreversibly and reversibly to MHC and TCR, respectively, to stimulate a T-cell response.

Drug metabolites and drug-induced humoral immunity

Although this review focuses on T-cell-mediated drug allergies, cellular and humoral immunity should not be considered in isolation as they often develop together as part of a multifaceted immune response to antigen exposure.

Even though studies on humoral immunity in drug-induced T-cell hypersensitivity reactions are limited, both antidrug and antitissue antibodies have been detected in individuals allergic to drugs, including SMX [88,89], diclofenac [90,91], lidocaine [92], or carbamazepine [93,94]. Such antibodies are thought to be secondary to an immune response to drug-protein adducts formed by reactive drugs such as penicillins, or by reactive metabolites such as nitroso-SMX. The binding of the drug to the protein would create a neoantigen unknown by the immune system, which could trigger a reaction against the drug antigen itself, and to the protein itself, and both.

Because the extent of research on drug-induced humoral immunity is quite limited in delayed drug hypersensitivity, it remains unknown whether these antibodies are

simple markers of the reaction or they could be a pathogenic cofactor in T-cell-mediated drug hypersensitivity. However, the pathogenic role of antibodies is accepted in several delayed drug allergies. For instance, serum sickness-like syndromes are thought to be triggered by the tissue deposition of antibody–antigen immune complexes. In the case of cefaclor-induced serum sickness-like reactions in children, Kearns *et al.* [95] even showed that drug metabolism is important in the pathogenesis of the reaction.

Pharmacogenomics and drug metabolites

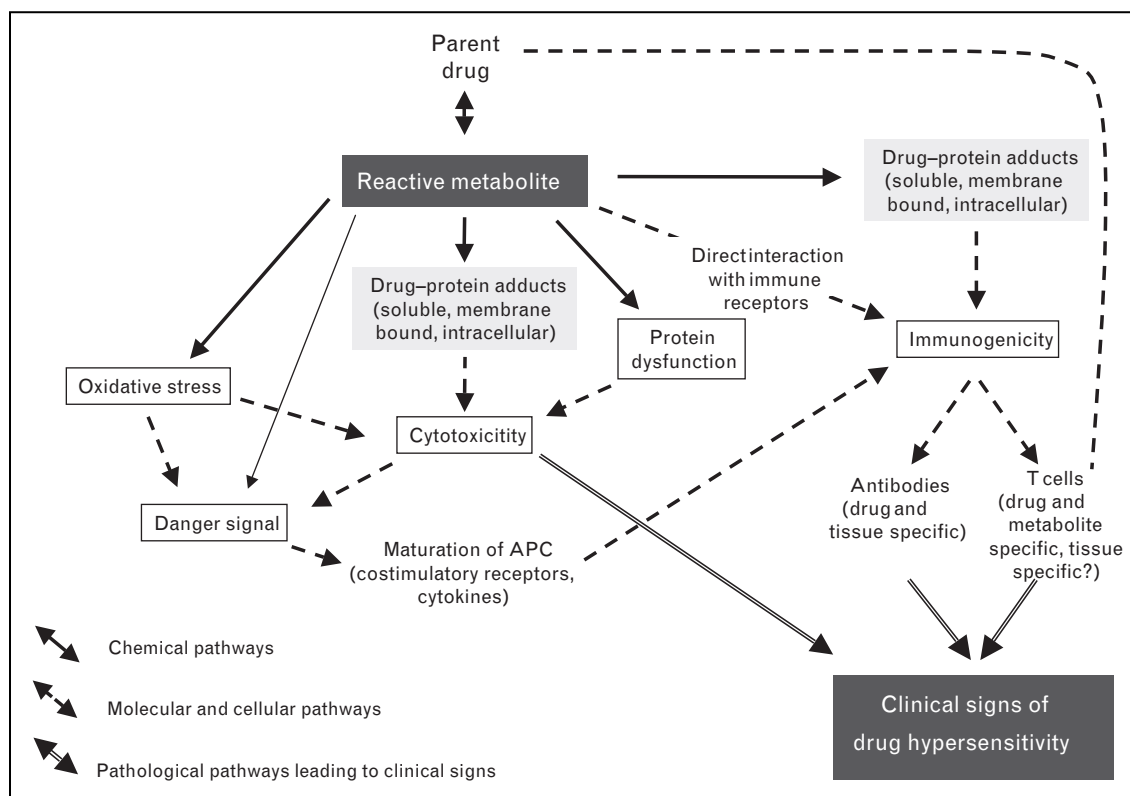
For the past decade, a strong emphasis has been put on studying the correlation of drug hypersensitivity with HLA polymorphisms. Correlations have been identified with certain HLA alleles and hypersensitivity with carbamazepine [96,97], abacavir [98,99], allopurinol, or ximelagatran [100]. To date, nobody has investigated polymorphisms of TCR as a risk factor in T-cell-mediated drug allergies.

In parallel, a few pharmacogenomics studies have investigated polymorphisms in drug-metabolizing enzymes in drug hypersensitivity [101]. They were based on the assumption that, under the prohapten theory, some genetic polymorphisms could increase the formation of reactive metabolites, and therefore increase the risk of drug hypersensitivity. Although potential pathways of metabolic bioactivation have been investigated in greater detail than detoxification pathways, there is some evidence suggesting that polymorphisms in detoxification enzymes could play a role in susceptibility to drug hypersensitivity (e.g. SMX and cytochrome b5/cytochrome b5 reductase pathway [102*]).

Conclusion

The multiple potential roles of reactive metabolites in the generation of drug-specific immunity, both humoral and cellular, are highlighted in Fig. 2. Although significant progress has been made in our understanding of metabolic processes and antigen-specific immune

Figure 2 Potential roles of drug metabolites in the pathogenesis of drug hypersensitivity



Some drugs are metabolized to known reactive metabolites. This chemical reaction itself, or the detoxification of the reactive metabolite, can generate some reactive oxygen species and deplete the oxidant pool, which are a source of oxidative stress for the cell. Some drug metabolites have been shown to stimulate the innate immunity, which could further lead the immune system towards an active reaction rather than tolerance of the drug. Some reactive metabolites can also bind to proteins. Such drug–protein adducts can be safe, toxic (preventing some fundamental protein function), or immunogenic. The subsequent humoral and T-cell immune reaction, associated with some drug metabolism-induced oxidative stress, could lead to tissue damage, and explain the clinical signs associated with drug allergy.

responses, our knowledge remains incomplete in most cases. First, more work will be necessary to determine what differentiates a toxic and immunogenic drug–protein adduct from a protective one: nature and function of the protein; localization of the protein (extracellular vs. membrane vs. intracellular); nature and number of amino acids involved. Additionally, the determination of the exact nature of the antigen recognized by the T cell (free drug vs. drug–protein hapten; parent drug or metabolite, nature of the peptide carrier) will also require further investigation. Moreover, it will also be important to investigate any direct pharmacological interaction between drug metabolites and immune receptors, within the framework of the pi concept. Finally, focus should be directed towards the disposition of parent drugs by dendritic cells, including bioactivation, and its downstream consequences on the lymphocyte response.

In conclusion, some clinical and laboratory evidence exist to justify all theories behind drug hypersensitivity (Table 1). Future interdisciplinary collaborative research will probably show that all hypothesized mechanisms (prohaptent hypothesis, pi concept, drug-induced humoral immunity, danger theory) coexist *in vivo* to lead to drug-induced clinical signs, with some pathways being more important than others depending on the individual (e.g. genetic background, infection) and the drug, leading to different tissue targets (skin vs. liver vs. blood cells...) and different severity levels (e.g. simple skin rash vs. Steven–Johnson syndrome).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 362–363).

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