

Drug skin metabolites and allergic drug reactions

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Purpose of review

Presentation of recent studies about xenobiotica metabolism of the skin and its impact on drug-allergic reactions.

Recent findings

The skin possesses not only multiple cytochrome P450 isoenzymes but also influx and efflux transporter proteins. The pattern of cytochrome P450 isoenzymes in the skin differs from the pattern in the liver. Studies on the role of this metabolism in drug-induced hypersensitivity have mainly focused on sulphonamides, anticonvulsants, diclofenac and nevirapine. Studies with the contact sensitizer carboxime provided evidence for the decisive role of xenobiotica metabolism in antigen-presenting cells.

Summary

The skin is a major target organ for allergic drug reactions that may be explained by its different barrier functions including immune system and the armamentarium of xenobiotica-metabolizing enzymes.

Keywords

contact sensitizer, cytochrome P450, drug allergy, sulfamethoxazole

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Introduction

The skin is a major target organ for allergic reactions to small molecular weight compounds. Examples include drug-allergic reactions that can present with many different clinical manifestations including bullous drug reactions such as Stevens–Johnson syndrome or toxic epidermal necrolysis, although morbilliform drug rashes are the most common [1]. Another example is allergic contact dermatitis that can be induced by topically applied drugs. However, a multitude of other chemical compounds can also elicit similar reactions [1]. The sensitization process that finally leads to allergic contact dermatitis has the following features that are in common with allergic drug reactions: they are induced in more than 90% by small molecular weight compounds, and allergic contact dermatitis as well as drug-induced exanthemas, which are, by far the most common type of allergic drug hypersensitivity reactions, delayed-type allergic reactions. Thus, lessons on the pathophysiology of these adverse drug reactions can be learnt from studies with contact sensitizers [1].

There are two main reasons for the observation that the skin is a major target organ for these allergic reactions. Firstly, the skin possesses antigen-presenting dendritic cells that are able in particular to elicit hypersensitivity reactions (in contrast to those in the gastrointestinal tract). Secondly, the skin possesses the armamentarium

to metabolize xenobiotica to highly reactive species that are able to bind to high molecular weight compounds, which may lead to sensitization via the formation of haptens. In this review, we will summarize the recent evidence with regard to skin as a xenobiotica-metabolizing organ, studies on the role of metabolism in allergic reactions to sulphonamides, diclofenac, anticonvulsants and nevirapine, and finally some conclusions from studies with contact sensitizers that might be relevant to the pathogenesis of drug hypersensitivity reactions.

Skin as a xenobiotica-metabolizing organ: the second barrier of the skin

Recent studies have demonstrated that the skin is an active site of drug and xenobiotica metabolism. The skin possesses a broad spectrum of catalytic activity mediated by a diverse array of metabolizing enzymes. In particular, recent research results have shown that human skin keratinocytes express various transport-associated and P450-dependent metabolic enzymes, and therefore provide the capability for active uptake, biotransformation and antitransport of a range of xenobiotics including drugs, solvents and carcinogens [2]. Apart from the role of the stratum corneum as critical for epidermal structural barrier function, there is increasing evidence for the concept that xenobiotica-metabolizing enzymes and transport proteins can function as a second biochemical barrier for the skin [3]. This second biochemical barrier of

the skin mediates a xenobiotica metabolism that can be divided into at least three phases: phase 1 involving the activation of the parent compound by oxidizing enzymes, in particular cytochrome P450 (CYP)-dependent enzyme activities, to highly reactive intermediates; phase 2 in which the highly reactive intermediate may be metabolized by other enzymes, such as transferases, to more water-soluble metabolites that can leave the cells; and finally phase 3 that is mediated by influx and efflux transporter proteins that have been shown to be present in cutaneous cells such as keratinocytes and antigen-presenting dendritic cells (Fig. 1). However, if there is an imbalance between these three phases of xenobiotica metabolism in the cell, the highly reactive intermediates can also bind to high molecular weight molecules, such as proteins, in order to induce toxic responses including sensitization (Fig. 1). Members of all xenobiotica-metabolizing CYP isozyme families have been characterized on the RNA, protein and catalytic level, in particular those of the *CYP 1*, *CYP 2* and *CYP 3* gene family [4^{••},5[•]]. Although several hepatic CYPs are expressed in the skin (e.g. CYP 2E1), there are in addition other cutaneous CYPs not expressed in the liver, which may lead the relative proportion of metabolites produced in the skin to differ from those in the liver [6] (Table 1) [7]. In order to reflect these differences, we recently developed a specific skin cocktail of CYPs known to be present in the skin [7]. In Table 1, a classical liver CYP cocktail is compared with the skin CYP cocktail. CYP 2E1 is present in the liver as well as in the skin, and its concentration equals its amount in the liver cocktail, so that the other CYPs were increased according to their presence in the skin [7].

Table 1 Comparison of cytochrome P450 isoenzymes present in the liver versus skin cytochrome P450 cocktail

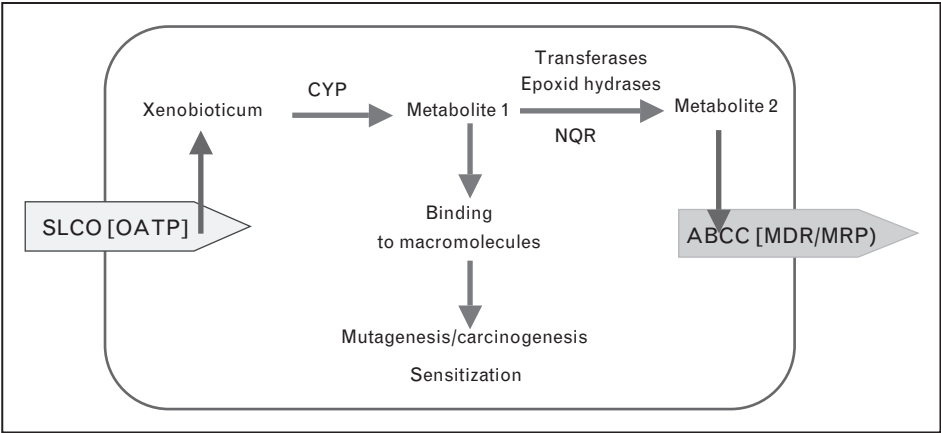
CYP isoenzyme	Liver (pmol)	Skin (pmol)
1A1	–	3.6
1A2	20	–
1B1	–	2.0
2B6	–	0.035
2C9	17	–
2C19	8	–
2D6	3	–
2E1	11	11
3A4	45	–
3A5	–	5.6
Total	104	22

CYP, cytochrome P450. Data from [7].

Sulphonamides

Drug-allergic reactions to sulphonamides were studied as one example of a drug that is metabolized. Sulphamethoxazole (SMX)-specific T-cells in a patient with a bullous exanthema revealed a significant proliferation of SMX and autologous irradiated lymphocytes as antigen-presenting cells *in vitro* only after addition of SMX-modified murine liver microsomes that were rich in drug-metabolizing CYP enzymes. The induction was shown, despite the fact that oxidized sulfonamides and their metabolites revealed an immunosuppressive activity in T-cells [8,9]. Further studies [10,11] revealed the presence of antibodies against protein–sulfonamide compounds in patients with late-type sensitizations to sulfonamides. Glucose-regulating protein 78 (grp78) and the protein disulfide isomerase could be identified as the binding structure in the endoplasmic reticulum [11].

Figure 1 The skin possesses an armamentarium of enzyme-mediated xenobiotica metabolism, including cytochrome P450 isoenzymes, enzymes of the phase II reaction such as epoxide hydrases, reduced nicotinamide adenine dinucleotide phosphate quinine reductases, transferases as well as influx and efflux transporter molecules



Phase I, CYP; phase II, epoxide hydrases/transferases; phase III, transporter proteins. In addition, it was found that small molecular weight compounds are transported into and out of a cell by specific transporter molecules. Their expression is closely linked to the expression of CYP enzymes. ABCC, ATP-binding cassette C transporters; CYP, cytochrome P450; MDR, multidrug resistance; MRP, multidrug resistance protein; NQR, nicotinamide adenine dinucleotide phosphate quinine reductases; OATP, organic anion transporting polypeptide; SLCO, solute carrier organic anion transporter.

Although these studies did not reveal the pathophysiological role of these antibodies as a cause of or epiphenomenon in the reaction, they underline the importance of the oxidative, CYP-dependent metabolism in sensitization of a patient. The hapten theory that underlies the impact of drug metabolism as a prerequisite for many drug-allergic reactions was challenged by the p-i concept. Studies on T-cell clones isolated from patients sensitized to a drug revealed that the parent compound in question was able to activate sensitized T-lymphocytes directly. These results refuted earlier findings that oxidative metabolism of the drug is necessary to develop antigens. This type of reaction is found in sensitization to local anesthetics, anticonvulsant drugs, sulfonamides and, in our own studies, with contact sensitizers such as *p*-phenylenediamine [12,13]. These observations supported the p-i principle, a direct T-lymphocyte reaction to such substances without prior processing by an antigen-presenting cell [12]. However, one has to bear in mind that most T-cell clones examined reacted to the drug as well as to its metabolite(s). In addition, studies with T-lymphocytes from already sensitized individuals may not reflect the process of primary sensitization. Recently, the effects of SMX and its toxic nitroso metabolite on costimulatory signals of dendritic cell maturation were studied. In this study, at the level of dendritic cells, the authors demonstrated an increased CD40 expression but not CD80, CD83 or CD86 with dendritic cell surfaces exposed to SMX and the protein-reactive metabolite nitroso SMX. This reaction that was not associated with apoptosis, necrosis or glutathione depletion was higher with the nitroso SMX metabolite than with SMX. In addition, the CYP-inhibiting 1-aminobenzotriazole prevented the increase of CD40 expression by SMX, whereas the reaction to the nitroso derivative was not inhibited if one compares these results with controls without the inhibitor 1-aminoazotriazole [14].

Diclofenac

Another recently investigated example is diclofenac. Diclofenac can induce anaphylactic reactions in patients with aspirin intolerance (nonsteroidal anti-inflammatory drug cross-reactivity) or by drug-specific allergic reactions. Furthermore, delayed-type allergic reactions after systemic administration, as well as contact dermatitis after topical application can occur. Recent studies in mice demonstrated that sensitization to diclofenac is induced by a quinoneimmin derivative of diclofenac, a CYP 3A4-dependent derivative [15].

Anticonvulsant drugs

Anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigine or benzodiazepam are metabolized to highly reactive arene oxide metabolites by CYP-dependent

enzymes and can be detoxified by epoxide hydrolases. Alternatively, cytotoxic intermediate metabolites can bind to a protein and therefore become immunogenic, and a lymphocyte toxicity assay has been developed to help determine phenytoin sensitization *in vitro* [16]. In addition, the lymphocyte reaction *in vitro* (lymphocyte transformation test, LTT) to phenytoin and carbamazepine could be enhanced after addition of liver microsomes with CYP activity to the LTT [17,18]. In the case of benzodiazepam derivatives, the addition of microsomal protein, which contains most of CYP activity, leads to an increase in T-lymphocyte proliferation as well as IL-5 release by the lymphocytes [19]. It was also shown that skin expresses CYP isoenzymes capable of metabolizing carbamazepine into reactive metabolites that bind covalently to proteins [20]. Finally, antibodies to CYP 3A4, the enzyme metabolizing anticonvulsant drugs, could be detected in patients with sensitization to anticonvulsive drugs. T-cell clones from these sensitized patients reacted to metabolites of carbamazepine [21]. Lymphocytes and T-cell clones were isolated from patients with hypersensitivity reactions to carbamazepine and oxocarbamazepine. They proliferated with the parent compound as well as with 10,11-epoxide and 10-hydroxy-derivatives [22]. 1-aminobenzotriazole inhibits CYP activity but did not inhibit the T-cell proliferation to the parent compound, supporting the similar observation with sulphonamides or *p*-phenylenediamine that T-lymphocyte reactivity of sensitized individuals reacts differently compared with the interaction of the immune system and small molecular weight compound during the phase of primary sensitization [22]. In animal experiments, the same authors provided evidence that highly reactive metabolites of carbamazepine are the nominative antigens [23].

Nevirapine

To diagnose sensitizations to nevirapine, an animal model has been developed [24]. Interestingly, this model provided evidence that a reactive metabolite induces the immune response, but once induced, T-cells are generated that also respond to the parent drug [24]. In particular, nevirapine is metabolized by CYPs to 2,3 and 12-hydroxy derivatives. The addition of 1-aminoazotriazole, which inhibits most of CYP isoenzymes, prevents the formation of the 2-hydroxy and 3-hydroxy metabolites but not the formation of the 12-hydroxy metabolite. The 12-hydroxy metabolite is further metabolized by sulfotransferases, which are known to be present in skin to an intermediate that is subsequently converted spontaneously to a most reactive quinone methide derivative that is most likely the nominative antigen [24]. However, T-lymphocytes of sensitized and rechallenged animals produce IFN γ in response to the parent compound as well as to the 12-hydroxy metabolite [24].

Lessons from allergic contact dermatitis

The characterization of CYPs, which are present in the skin, allowed us to prepare a skin-like recombinant human CYP cocktail that is similar to the CYPs in the skin under in-vivo situations [7]. The skin-like CYP cocktail also allows increased enzymatic activity in order to obtain enough metabolites for the subsequent chemical analysis.

The first compound that has been investigated with such a system was a diene, which according to in-silico skin sensitization tests, should not be a sensitizer. However, under in-vivo conditions in the murine lymph node assay, it was ruled out to be a moderate skin sensitizer. Hence, it has been speculated that in the in-vivo situation, a metabolism to endocyclic or exocyclic derivatives may occur and may explain these contradictory results. Consequently, the parent compound as well as the metabolites were incubated with human monocyte-derived dendritic cells (moDCs), and it was only the exocyclic epoxide derivative that strongly induced IL-8, which can be thus be used as a marker for dendritic cell activation by antigens, suggesting that this CYP-dependent metabolite is the nominative antigen [7].

Recently, the prohaptens carboxime was also studied using this system. This compound is metabolized by different CYPs to a metabolite 2 and metabolite 3. Only CYP 1A1 is involved in the formation of metabolite 2, which is produced by CYP 1A1 and CYP 1B1, which is primarily expressed in dendritic cells. Interestingly, in the murine lymph node assay, metabolite 2 was ruled out to be recognized as an antigen after sensitization with the parent compound but not the metabolite 3. Even more interesting is the observation that the parent compound strongly induces CYP 1B1 in dendritic cells without having any effect on the CYP 1B1 expression in keratinocytes.

Obviously, the parent compound carboxime paths the metabolic pathway to the metabolite 2 that seems to be the sensitizer, because this metabolite is produced by CYP 1B1 [25^{*}]. This observation suggests that the CYP-dependent metabolism in antigen-presenting cells might be decisive during the sensitization process compared with the metabolism in keratinocytes or liver cells, because CYP 1B1 is not a major representative of the CYP1 family in keratinocytes and the liver.

Conclusion

Skin possesses properties as a physical, immunological as well as biochemical barrier that seem to be important prerequisites for the observation that the skin is a major target organ for drug-allergic reactions. In particular,

recent studies on the interaction between small molecular weight xenobiotics and the immune system in the development of allergic contact dermatitis provide new insights into the pathophysiology of these allergic reactions and may provide important lessons for the understanding of drug hypersensitivity reactions. The p-i concept as well as the hapten concept seem to be operative in challenges of already sensitized patients. Therefore, these concepts have to be taken into consideration, for example, in in-vitro diagnostic procedures such as the LTT.

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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. 000–000).

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