



Review

Skin: Major target organ of allergic reactions to small molecular weight compounds

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Received 16 November 2006; revised 5 January 2007; accepted 9 January 2007

Abstract

Skin is a major target organ for allergic reactions to small molecular weight compounds. Drug allergic reactions may be life-threatening such as in the case of anaphylactic reactions or bullous drug reactions and occur in about 5% of all hospitalized patients. Allergic contact dermatitis has an enormous influence on the social life of the patient because it is the most frequent reason for occupational skin diseases and the treatment and prevention of this disease cost approximately € 3 billion per year in Germany.

The different proposed pathophysiological pathways leading to a drug eruption are discussed in this paper. All major enzymes which are involved in the metabolism of xenobiotica were shown to be present in skin. Evidence supporting the role of metabolism in the development of drug allergy and allergic contact dermatitis is demonstrated in the example of sulphonamides and fragrances.

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Keywords: Drug allergy; Contact dermatitis; Skin metabolism; Prohaptens; Xenobiotica

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Introduction

Skin is a major target organ for allergic reactions to small molecular weight compounds. These allergic reactions can be precipitated as urticaria, angioedema, purpura, vasculitis, exanthema, or bullous drug reactions such as fixed drug eruption, erythema exudativum multiforme, Stevens-Johnson-Syndrome, or even toxic epidermal necrolysis. Drug allergic

reactions may be life-threatening and occur in about 5% of all hospitalized patients. Up to 81% of all hospitalized patients suffer from pharmaceutical side effects and 26% of all adults suffer from an allergic or pseudoallergic drug eruption during the course of their lives (Friedman et al., 1994; Greenberger, 2006; Weiss, 1992).

Allergic or hypersensitivity reactions are the result of an immune response and therefore require a sensitization of the immune system followed by the formation of specifically reacting T-lymphocytes or antibodies such as IgE-antibodies in the case of anaphylaxis. Idiosyncrasy describes a qualitatively abnormal,

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unexpected response to small molecular weight compounds such as drugs, differing from its pharmacological actions and therefore may resemble hypersensitivity (DeSwarte and Patterson, 1997). In this review, we will focus on hypersensitivity or allergic reactions to small molecular weight compounds.

In a prospective study over the time period of 6 months, undesirable skin reactions to drugs were seen in 3.6/1000 hospitalized patients (Fiszenson-Albala et al., 2003). The best epidemiological data exist for anaphylactic reactions to β -lactam antibiotics. Various studies report between 0.7 and 0.8% allergic reactions due to β -lactam antibiotics, including anaphylaxis. Deadly incidents due to anaphylaxis were reported in 1 in 50,000 to 1 in 100,000 treatment cases. In the U.S., 400–800 deaths/year were caused by anaphylaxis (Weiss, 1992). Retrospective, population-based studies of bullous drug allergies reported an incidence of bullous drug reactions in 1.2/1,000,000 inhabitants/year in France and 0.93/1,000,000 inhabitants/year in Germany (Mockenhaupt, 2005).

Autoimmune diseases and infections are a risk factor to adverse drug reactions. This is especially true in HIV-infections. Eighty percent of HIV positive patients receiving trimethoprim and sulfamethoxazole develop a drug-related allergy, while only 1–3% of HIV-negative patients develop an allergy to trimethoprim and sulfamethoxazole (Gruchalla and Pirmohamed, 2006). Similarly, 30% of patients suffering from cystic fibrosis develop a sensitization to piperacillin, cefoxitin, or ticarcillin, while infectious mononucleosis predisposes patients to developing an amoxicillin induced drug rash (Gruchalla and Pirmohamed, 2006).

Allergic contact dermatitis is normally not a life-threatening disease. However, it is the most frequent reason for occupational skin diseases and has an enormous influence on the patient's social life. Therefore, in Germany the health assurance system for the treatment and prevention of occupational diseases ("Berufsgenossenschaften"), spends around € 3 billion per year for the treatment and prevention of allergic contact dermatitis.

Skin possesses several immunocompetent cells like the antigen-presenting Langerhans cells as well as xenobiotic metabolizing enzymes that process chemical compounds into chemically active components. It is proposed that the combination of these two mechanisms makes the skin a target organ for allergic reactions to small molecular weight compounds.

Xenobiotica metabolizing enzymes of the skin and their role in allergic reactions to small molecular weight compounds

A small molecular hapten by itself cannot be recognized as an antigen. In order to be recognized and processed as an antigen, it needs to be attached to a peptide or protein. Drugs and other xenobiotics entering the body will be activated by metabolic conversion. The enzymes involved in this metabolism are cytochrome P450 (CYP) isoenzymes, glucuronosyl transferase, flavin-containing monooxygenase, *N*-acetyl-transferase and monoamine oxidase (Guengerich, 2006).

It is proposed that CYP enzymes are involved in the pathophysiology of allergic drug reactions. One evidence for

this involvement is the observation that in many allergic drug reactions, liver–kidney–microsomal antibodies are detected that bind to CYPs located at the microsomal membrane (Beaune et al., 1987; Cribb et al., 1997). CYPs are not only located in the liver, but are also present in the skin—the main target organ of drug allergic reactions. Therefore the extrahepatic metabolism in the target organ may be important (Ahmad et al., 1996; Ahmad and Mukhtar, 2004).

Studies on enzymatic dependent metabolism of xenobiotics in extrahepatic tissues revealed a monooxygenases-dependent catalytic activity in murine and human skin. The catalytic activity could be induced and/or inhibited by different xenobiotics including drugs (Bickers and Kappas, 1978; Bickers et al., 1982; Levin et al., 1972; Merk et al., 1987). The presence of multiple CYP enzymes in the skin was shown on an mRNA and protein levels (Baron et al., 2001). Reverse transcription-polymerase chain reaction (rtPCR) revealed constitutive expression of cytochromes 1A1, 1B1, 2B6, 2E1, and 3A5 in keratinocytes and showed an expression of cytochrome 3A4 after incubation with dexamethasone. The expression of cytochrome 1A1 was enhanced on the mRNA level after induction with benzanthracene (Baron et al., 2001; Du et al., 2006). mRNA data were confirmed on the protein level by immunoblots and immunohistology which showed expression of cytochrome P450 1A1, 2B6, 2E1, and 3A. Constitutive activity of CYP 1A1, 2B, 2E1, and 3A enzymes was measured by catalytic assays (Baron et al., 2001). Also flavin-containing monooxygenases were found in normal human epidermal keratinocytes (NEHK)—in particular the FMO3 (Janmohamed et al., 2001; Vyas et al., 2006a, 2006b). Furthermore, transferases mediating the second phase of xenobiotic metabolism such as glutathione-*S*-transferases, *N*-acetyl-transferases, or UDP-glucuronosyl transferases were detected in the skin (Kawakubo et al., 2000; Lilienblum et al., 1986; Mukhtar and Bresnick, 1976).

Further studies showed that monooxygenases and transport-associated proteins such as multidrug resistance-associated transport proteins (MRPs) play complementary parts in drug disposition by biotransformation (phase I) and anti-transport (phase III) and act synergistically as a drug bioavailability barrier. RtPCR analysis of membrane-associated transport proteins revealed constitutive expression of MRP 1 and 3–6 in human epithelial keratinocytes, demonstrating that normal human keratinocytes express a cell-specific pattern of efflux transport proteins (Baron et al., 2001). Using real-time PCR, RT-PCR, cDNA microarray, immunostaining and efflux assays, it was shown that stimulation of NHEK and primary human dermal fibroblasts with interleukin-6 (IL-6), in combination with its soluble alpha-receptor, or oncostatin M (OSM) resulted in an upregulation of MRP expression and activity (Dreuw et al., 2005). These results suggest a regulating control between the transformation of small molecular weight compounds and inflammatory skin disorders.

In addition, expression of transport proteins involved in the uptake of xenobiotics has been detected on the mRNA and protein levels in human keratinocytes, revealing a different expression pattern as compared to primary liver cells (Schiffer

et al., 2003). The transporters studied included the subtypes A, B, C, D, and E of the organic anion transporting polypeptide (OATP) family, which are responsible for the uptake of various anionic and neutral molecules and especially organic cations, including drugs. Constitutive expression of OATP-B, OATP-D, and OATP-E was shown in NHEK using RT-PCR and northern blot analysis, as well as in human skin tissue shown by tissue blot hybridization and immunohistochemistry (Schiffer et al., 2003). Expression of OATP-A and OATP-C was not detected in any of the keratinocyte samples. In contrast, liver tissue showed a significant expression of OATP-A and OATP-B as well as OATP-C, a weak expression of OATP-D, and no expression of OATP-E (Schiffer et al., 2003). Using an uptake-transport assay, uptake of known and well-characterized OATP substrates like estradiol-17 β -glucuronide and estrone sulfate was inhibited in normal human epidermal keratinocytes by specific inhibitors such as taurocholate, verifying the functional capacity of the expressed OATPs. Even though the substrate specificity of the OATP isoforms is only partially known, these findings support the concept that uptake of large organic cations like drugs or allergic contact dermatitis antigens in keratinocytes is an active transport process mediated by members of the OATP family.

These data confirm that NHEK expresses a specific profile of xenobiotica-metabolizing enzymes and transporters involved in drug influx and efflux. This may be important for the processing and presentation of small molecular weight compounds including drugs like carbamazepine, sulfonamides, or contact allergens such as fragrances (Wolkenstein et al., 1998; Reilly et al., 2000; Sieben et al., 2001; Merk et al., 2002).

Sulfonamides

The sulfonamides are antibiotics which are associated with serious hypersensitivity adverse reactions including bullous drug allergic reactions such as TEN. We isolated lesional lymphocytes from a patient who suffered from a drug-induced allergic erythema exudativum multiforme. Interestingly, these T-lymphocyte clones only reacted to sulfamethoxazole after incubation with appropriate antigen-presenting cells, sulfamethoxazole, and liver microsomes which possess CYP-dependent enzyme activity (Hertl et al., 1995). Sulfonamides are metabolized by *N*-acetyltransferases to acetylated derivatives which are not sensitizing. However, alternatively sulfonamides such as sulfamethoxazole are activated by CYPs to arylhydroxylamine derivatives which can be converted spontaneously to nitroso sulfamethoxazole metabolites (Naisbitt et al., 2002; Vyas et al., 2006a). The incubation of NHEK with the arylhydroxylamine metabolites of sulfamethoxazole resulted in the formation of metabolite-protein adducts (Reilly et al., 2000). Furthermore, confocal laser microscopic studies reveal covalent binding of sulfonamide-metabolite-protein to the membrane of keratinocytes in close proximity of the HLA-ABC proteins (Roychowdhury et al., 2005). More recent studies suggest that most likely FMO3 plays an important role in the bioactivation of sulfonamides in NHEKs. Methimazole, a prototypical substrate of the FMOs, attenuated the protein haptenation observed in NHEKs exposed to sulfamethoxazole or dapsone.

Inhibitors of CYPs such as aminobenzotriazoles and 1-dichloroethylene did not reduce protein haptenation of sulfamethoxazole (Vyas et al., 2006a, 2006b).

Pichler et al. proposed an alternative pathomechanism leading to allergic drug reactions (Pichler, 2004). Studies on cloned T-cells revealed that they may be stimulated by glutaraldehyde-fixed AG-presenting cells. No activation of T-cells occurred after short pulsing with small molecular weight compounds such as sulfamethoxazole, lidocaine, carbamazepine, lamotrigine, phenindione, and *p*-phenyldiamin (Naisbitt et al., 2005; Sanderson et al., 2006a, 2006b; Sieben et al., 2002). These results suggest that T-cell activation to small molecular weight compounds is possible without prior antigen processing and metabolism to highly reactive species which are needed for covalent binding. This proposed pathomechanism may be important for immune response after sensitization and therefore may have an impact on the diagnosis of drug allergic reactions. However whether this pathomechanism plays a role in sensitization to small molecular weight compounds is open. In the murine system, sensitizations were only inducible with oxidized metabolites (Hopkins et al., 2005; Naisbitt et al., 2003, 2005).

A model for nevirapine sensitization showed that metabolites other than those detected by T-cells might play a role in drug sensitization (Chen et al., 2006; Popovic et al., 2006). This model revealed that sensitization was primarily induced by a cytochrome P450-dependent 12-hydroxy-metabolite of nevirapine. Interestingly, the active pharmaceutical ingredient (API), but not the metabolite was cognized by T-cell clones isolated after sensitization. Thus, sensitization might not only be caused by metabolites of an API but also by the API itself. APIs that are recognized by sensitized T-cells do not necessarily reveal the conditions leading to the sensitization (Popovic et al., 2006).

Fragrances

In most western countries, sensitization rates are highest to nickel, followed by fragrances (De Groot and Frosch, 1997). Their ubiquitous distribution leads to unavoidable exposure. Fragrances are not only present in cosmetic products but also in household products, medications, food, paper, and paints. There are several examples where fragrances are oxidized to become the nominative antigen. This may occur by spontaneous oxidation as in the case of D-limonene, linalool (Karlberg et al., 1994, 1988; Matura et al., 2002; Sköld et al., 2004), or by enzymatic metabolism, for example, cinnamic alcohol or eugenol (Basketter, 1992; Bertrand et al., 1997; Sieben et al., 2001; Smith et al., 2000). Sieben et al. have observed that the T-cell stimulation of eugenol- or isoeugenol-sensitized patients was increased in the presence of antigen-modified human liver microsomes (CYP) or recombinant CYP 1A1 in five of seven cases (Sieben et al., 2001). This suggests that metabolically active enzymes such as CYPs may be involved in the activation of fragrances and that the study of those enzymatic pathways may be important e.g. to recognize potentially strong antigens by appropriate in vitro cell systems or knowledge-based expert systems such as TOPS-MODE (topological substructural

molecular descriptors) in combination with DEREK, which represents the knowledge archive (Estrada et al., 2004).

Recently we studied a diene which was predicted by expert systems as a non- or low-sensitizing compound. However in sensitizing experiments in mice and guinea pigs revealed it to be a strong sensitizer (Nilsson et al., 2005; Andresen Bergström et al., 2006). The incubation of this compound with CYP enzymes present in the skin resulted in the formation of epoxide derivatives (Bergström et al., 2007). This prompted us to establish a skin-specific CYP cocktail which reflected the relative proportion of different CYP enzymes present in the human skin (Bergström et al., 2007). Incubation of human monocyte-derived antigen presenting cells with the compounds or its epoxide derivatives revealed especially the exocyclic epoxide metabolite and, to a lesser extent, the diene-induced expression of proinflammatory cytokines including interleukin 8 in these cells. This prediction regarding the sensitizing properties of these derivatives was mirrored in the local lymph-node assay in mice which also revealed the epoxide derivatives as the main sensitizers (Bergström et al., 2007).

Outlook

Currently, three developments are under investigation to explain pathophysiology of allergic reactions to small molecular weight compounds. These approaches might influence our diagnostic and therapeutic abilities:

1. Establishment of animal models enable examination of the primary sensitization phase – as applied in diagnostics of sensitized patients – and also comparison of the secondary inducers of the reaction as well as analysis of sequences of allergic reaction (Nierkens and Pieters, 2005; Popovic et al., 2006; Shenton et al., 2004; Uetrecht, 2006). These models demonstrated the role of e.g. regulating T-cells in allergic drug reactions or autoimmune drug reactions (Layland et al., 2004; Masson and Uetrecht, 2004). Furthermore, these models allow the control of therapeutic concepts, like in TEN (Azukizawa et al., 2006).
2. The ability to use human monocyte-derived antigen-presenting dendritic cells creates the capability to test immunogenicity of small molecular substances. The metabolism of these substances can be altered by establishing artificial metabolizing systems (Bergström et al., 2007).
3. Recent pharmacogenetic studies have revealed individual risk factors such as HLA-association (Pirmohamed, 2006; Pirmohamed and Atuah, 2006; Alsbou et al., 2006). A better assessment of risk factors would enable the development of improved individual therapy and therefore contribute to an increase in drug safety. These studies might also increase our knowledge about pathophysiological mechanisms.

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