

Noncovalent Interactions of Drugs With Immune Receptors May Mediate Drug-induced Hypersensitivity Reactions

Submitted: August 26, 2005; Accepted: December 8, 2005; Published: March 17, 2006

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ABSTRACT

Drug-induced hypersensitivity reactions are instructive examples of immune reactions against low molecular weight compounds. Classically, such reactions have been explained by the hapten concept, according to which the small antigen covalently modifies an endogenous protein; recent studies show strong associations of several HLA molecules with hypersensitivity. In recent years, however, evidence has become stronger that not all drugs need to bind covalently to the major histocompatibility complex (MHC)-peptide complex in order to trigger an immune response. Rather, some drugs may bind reversibly to the MHC or possibly to the T-cell receptor (TCR), eliciting immune reactions akin to the pharmacological activation of other receptors. While the exact mechanism is still a matter of debate, noncovalent drug presentation clearly leads to the activation of drug-specific T cells. In some patients with hypersensitivity, such a response may occur within hours of even the first exposure to the drug. Thus, the reaction to the drug may not be the result of a classical, primary response but rather be mediated by existing, preactivated T cells that display cross-reactivity for the drug and have additional (peptide) specificity as well. In this way, certain drugs may circumvent the checkpoints for immune activation imposed by the classical antigen processing and presentation mechanisms, which may help to explain the idiosyncratic nature of many drug hypersensitivity reactions.

KEYWORDS: cross-reactivity, drug hypersensitivity, hapten, prohaptens, p-i concept, T-cell receptor, T cells

INTRODUCTION

Adverse effects to drugs are a common incidence for the clinician. Most reactions are caused by the pharmacological or toxicological activities of the drug and are generally

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predictable (type A). However, nonpredictable, idiosyncratic (type B) reactions^{1,2} may occur as well, amounting to ~15% of all cases. Most of the type-B reactions are mediated by the immune system and thus also termed drug-hypersensitivity reactions. Elicited by different immune mechanisms, they can become manifest as many distinct diseases.^{3,4} Often, the pathological mechanisms of these immune-mediated adverse effects are not completely clear. Some reactions are of the immediate type and are clearly mediated by antibodies.⁵ However, more recent studies by different groups clearly show that patients with drug hypersensitivity harbor drug specific T cells in their peripheral blood and in the affected tissues.⁶⁻⁸ The functions of these drug-specific T cells seem to determine the clinical picture of the disease.⁹

Drug-induced hypersensitivity reactions are fascinating diseases, as a small chemical compound can elicit a strong systemic immune reaction. These diseases can also be seen as “experimental models” of nature, with the physician performing an unintended—albeit instructive—“experiment.” By dissecting the underlying mechanisms, novel insights can be gained from these experiments not only about drug-hypersensitivity reactions in particular,¹⁻³ but also about immune reactions in general.

THE HAPTEN AND PROHAPTEN CONCEPT

How do small compounds such as drugs stimulate T cells? An answer to this question is certainly required to understand the side effects observed, and possibly allow for the prediction of these reactions based on the characteristics of the drug. For a long time, the “immunological dogma” postulated that small, low-molecular weight compounds per se are not capable of eliciting an immune response. In order for an immune reaction to occur, APC have to take up and process complex and large antigens and, subsequently, present these to T cells. However, small compounds such as drugs or metal ions were found to be able to trigger an immune response nevertheless. The hapten (and prohaptens) model is currently the accepted explanation for these observations. Chemically reactive, small compounds (ie, haptens) bind to proteins or peptides and modify them.¹⁰⁻¹² These are then processed and presented as hapten-modified peptides to T cells, which can react with the hapten antigen. Alternatively,

haptens may also bind to immune molecules that are involved in the presentation process, such as the major histocompatibility complex (MHC) itself.^{13,14} Prohaptens are a variation on the same theme: in order to become chemically reactive, they first need to be converted into a hapten by being metabolized into a compound that is chemically reactive.^{1,2,15,16}

EVIDENCE FOR THE EXISTENCE OF NONCOVALENT HAPTENS

The (pro)hapten concept elegantly circumvents the (presumed) blindness of the immune system for low molecular weight compounds by postulating that chemical reactivity and subsequent coupling to a macromolecule carrier is an absolute necessity. As a consequence, drugs and other substances that are incapable of such conjugation with a carrier would not be antigens and could not induce hypersensitivity reactions. However, there is clear clinical, immunological, and biochemical evidence to the contrary (Table 1).

The parental, not metabolized forms, of several different drugs are able to stimulate T cells via the T-cell receptor (TCR) in an MHC-dependent way, in particular lamotrigine,⁷ carbamazepine,⁸ sulfamethoxazole (SMX),^{17,18} mepivacaine,¹⁹ lidocaine,^{19,20} p-phenylendiamine,²¹ and radio-contrast media (RCM),^{38,39} even though most of them have known metabolites that can, in principle, act as haptens or are known to do so. SMX has been characterized particularly well, as its reactive metabolite SMX-nitroso (SMX-NO), acting as a typical hapten, was available for comparison.^{23,24} Hypersensitivity to the drug SMX was thought to be a consequence of bioactivation to the hydroxylamine metabolite (SMX-NHOH) and further oxidation to the ultimate, reactive metabolite SMX-NO. The antioxidant

glutathione is known to protect cells from reactive metabolites by conjugation and subsequent dissociation to SMX-NHOH and/or SMX.²⁴ However, only a minority of T-cell clones (TCC) derived from SMX-allergic patients reacted with the chemically reactive metabolite.²⁵ Most surprisingly, addition of glutathione to peripheral blood mononuclear cells enhanced rather than reduced the proliferation of T cells in response to SMX-metabolites,²⁶ presumably by transforming SMX-NO back to the "original" antigen, SMX. The response of SMX-NO-specific TCC was abrogated when glutathione was present during the covalent modification of APC. Collectively, these experiments support the concept that some T cells in allergic individuals recognize the noncovalently bound parent drug SMX rather than APC covalently modified by SMX-NO.^{25,26}

For several drugs, the kinetics of in vitro T-cell activation are simply much too fast for any involvement of antigen processing. In the presence of APC, lidocaine and SMX activate T cells quasi immediately as revealed by a rapid and sustained intracellular Ca²⁺ increase.¹⁸ It is impossible to reconcile this timing with an intermediate metabolism and processing step, which needs 60 minutes or longer to occur. Also, the kinetics of TCR down-regulation on drug reactive TCC after encountering the inert drug are similar to the recognition of preprocessed, immunogenic peptides (occurring within the first 30 minutes) and clearly differ from the recognition of proteins, which requires several hours.¹⁸ Several other observations argue against processing or covalent binding. For several drugs, specific TCC reacted even if the APC were fixed by glutaraldehyde, excluding the involvement of either processing or intracellular metabolism.^{7,8,17-19} Further, and at least for SMX, covalent binding is not necessary. Upon pulsing of APC,

Table 1. The p-i Concept: Evidence Pro and Contra*

PRO

- Numerous TCC specific for the parental form of several drugs despite the existence of reactive metabolites^{7,8,17-21}
- Glutaraldehyde-fixed APC can still present drug^{7,8,17-19,22}
- Washing removes drug and prevents T-cell activation^{17,22}
- Kinetics of TCR down-regulation too fast to allow antigen processing¹⁸
- Kinetics of Ca²⁺ mobilization too fast to allow antigen processing¹⁸
- Inhibition of SMX-NO generation by glutathione increases drug presentation²³⁻²⁶
- High incidence of unrestricted, drug-reactive clones²⁷
- Elevated frequency of alloreactive drug-reactive clones compared with peptide-specific TCC²⁸
- Exchange or removal of MHC-class-II-associated peptides does not affect drug presentation²⁹
- Kinetics of ERK phosphorylation too fast to allow antigen processing²²

CONTRA

- Clearly established for chemically reactive drugs¹⁰⁻¹⁴
- Delayed nature of majority of reactions; induction of a primary response is possible¹⁰⁻¹⁴
- Strong associations between several MHC-class-I alleles and drug hypersensitivity³⁰⁻³⁷

*TCC indicates T-cell clone; APC, antigen-presenting cell; TCR, T-cell receptor; SMX-NO, sulfamethoxazole-nitroso; MHC, major histocompatibility complex; and ERK, extracellular signal-related kinase.

which removes the drug (incubation of APC with the drug for 1 hour followed by 2 washing steps), no T-cell stimulation was observed, while the hapten SMX-NO, capable of covalently modifying the MHC peptide complex, was still able to stimulate hapten-reactive T cells.¹⁷ Many drug-specific TCC were found to be MHC-unrestricted,²⁷ and the frequency of alloreactive TCC is much higher among drug- than peptide-specific TCC from the same donor.²⁸ Last, the MHC-bound peptide seems to be irrelevant for SMX-specific T-cell activation.²⁹

Despite these unusual characteristics, T-cell activation by such drugs is TCR-dependent nevertheless, as recently shown using drug-specific TCR transfectants.²² Two SMX-specific human TCR were introduced into the mouse T-cell hybridoma cell line 54ζ17 (O. Acuto, personal communication, January 2003), according to the method described by Vollmer et al.⁴⁰ These transfectants expressed drug-specific TCR on the cell-surface and could be stimulated in a specific way in the presence of APC, resulting in interleukin 2 (IL-2) secretion. Key findings with these transfectants, which corroborated the previous observations with drug-specific TCC, were that the drug can be washed away (contrary to haptens covalently bound to carrier molecules), that the presence of APC (MHC) is required for IL-2 production, and that fixed APC are still able to present the drug. Similarly, the kinetics of TCR activation were too fast to involve antigen processing, as antigen-dependent extracellular signal-related kinase (ERK) phosphorylation was detected within 1 minute of SMX exposure.

THE P-I CONCEPT: THE TCR AS THE ANTIGEN BINDING MOLECULE?

Clearly, the hapten concept does not suffice to account for all the above observations. As a consequence, we have recently proposed a third model, which is not meant to contradict but rather to supplement the hapten/prohapten concept. Termed the p-i concept, which stands for “direct pharmacological interaction of drugs with immune receptors,”⁴¹ it states that certain drugs bind specifically and reversibly to some of the highly variable antigen-specific TCR in a direct way, instead of covalently modifying the MHC-peptide complex, which are the 2 feasible “partners” to accommodate allergy-inducing drugs. Such a drug-TCR interaction would be independent of metabolism and processing and, in fact, mimic drug interactions with other, nonimmunological receptors. While the MHC-peptide complex would not contribute (much) to the binding energy, it would still be necessary for full T-cell activation. Why do we think the TCR to be the more likely candidate for drug binding than the MHC, which is the “traditional” antigen-binding receptor?

The mere idea may appear far-fetched at first, but such a mechanism seems nevertheless feasible in principle, and at least one precedent has already been reported. Divalent Nickel ions (Ni) are generally considered haptens even though they do not bind to proteins covalently but rather by forming reversible coordination complexes.⁴² Weltzien and coworkers identified and characterized an HLA-DR-promiscuous, Ni-specific TCR in which Ni interacts simultaneously with the MHC and TCR by making contacts with a conserved His81 in the HLA-DR α -chain as well as Tyr29 and Tyr94 in CDR1 α of the TCR. Thus, Ni forms a bridge between both receptors, much like a superantigen, even though requiring idiotypic residues in the TCR.¹³ Ni has 6 coordination sites, of which only 3 are known for this complex at present. Nevertheless, a substantial part of its binding energy will be derived by the 2 (at least) contacts with the TCR of this complex. In fact, Ni binding may represent a “compromise” between how a typical hapten and a small antigen incapable of covalent binding may interact with the MHC and the TCR (see Figure 1 and legend for a detailed explanation).

For haptens, the majority of the antigen binding energy stems from the interaction with the MHC-peptide complex via few but strong covalent bonds (hapten; note that certain haptens may be strongly associated not only with the MHC but also the TCR⁴³). Ni may interact either like a noncovalent hapten¹⁴ or, as depicted here,¹³ forming equally strong, noncovalent interactions with both MHC and TCR (nickel), while at least some drugs would derive the majority of their binding energy from weak, noncovalent interactions with the TCR (p-i concept). These different modes of interaction represent a continuum of possibilities, with the (pro)hapten mode on one extreme of the spectrum, the p-i-concept mode representing the other extreme, and the Ni mode as an intermediate possibility.

Consider as well that $\alpha\beta$ TCR are peptide receptors. It has been known for 30 years that drugs can activate receptors that have peptides or proteins as endogenous receptors, the classical example being the opiate alkaloids. For these as

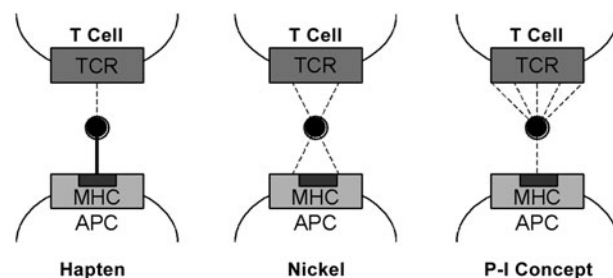


Figure 1. A schematic representation of how the TCR and the MHC might accommodate an antigen according to different models. The antigen (metal ion or drug) is depicted as a black ball, covalent bonds are indicated by bold lines, and noncovalent interactions by thin, dashed lines.

well as many other serpentine receptors, a myriad of compounds are known to bind and evoke many pharmacologically different responses. More recently, nonpeptide agonists have also been found for tyrosine kinases as well as growth factor and cytokine receptors.⁴⁴ However, apart from a hydrophobic cleft between the CDR3 α and CDR3 β regions, the TCR does not feature a “suitable” binding pocket or groove for small molecular weight compounds such as peptide receptors. Still, it cannot be excluded categorically that some drugs may bind to a particular TCR, especially given the huge TCR repertoire and the high level of cross-reactivity just from a probabilistic point of view alone.⁴⁵ It is also worthwhile to remember that the overwhelming majority of low molecular weight, “drug-like” compounds known to bind to differing receptor classes act as antagonists. In analogy to these findings, it seems likely that at least some drugs may not only activate but also block their (drug-specific) TCR.

THE P-I CONCEPT: DRUG REACTIVITY MASKING AS CROSS-REACTIVITY TO PEPTIDE ANTIGENS?

Even though the majority of drug-induced, T-cell-mediated skin reactions occur only after several days or even weeks of drug exposure, they can sometimes arise within a few hours after administration and/or without previous exposure to the drug (eg, documented for RCM).^{38,39} RCM are administered in extremely high doses, which might partly explain this observation. However, and notwithstanding the exceptional amounts of drug, the kinetics of such a reaction are much too fast to be explained by the induction of a classical, primary response, which is a prerequisite of the hapten model, as primary responses are mounted in the course of several days. On the other hand, a secondary response of the immune system is generally much faster and can lead to an immune reaction within the time frame observed for some adverse drug reactions. The existence of peptide-specific, preactivated memory T cells already present in the circulation and tissue that are cross-reactive to a particular drug seems an attractive explanation. If a sensitive individual harboring such cells were exposed to sufficient concentrations of the drug, these preactivated T cells would then be stimulated “accidentally” and induce a fast and potentially vigorous response. In line with this notion is the observation that the vast majority of drug-specific TCC have been found to bear $\alpha\beta$ TCR, which usually recognize peptides, and that a general stimulation of T cells, as in HIV infection,⁴⁶ is an important risk factor for drug hypersensitivity. Even more, it seems likely that drug-reactive cells exist even in individuals that are not hypersensitive: in an *in vitro* study, several blood donors who had never been exposed to SMX nevertheless harbored SMX- and SMX-NO-specific cells in their T-cell repertoire.⁴⁷

Hence, 2 (yet unproven) hypotheses are inherent to the p-i concept, and experiments unambiguously determining whether or not drug binding to TCR molecules occurs and if TCR double-specific for a drug and a peptide exist are currently under way. If these experiments proved the p-i concept to be correct, they would constitute an important step to show that it may truly be feasible to modulate T-cell-mediated responses in an antigen-specific way by using drugs or other low molecular weight compounds. If the p-i-concept is examined from a different angle, it becomes clear that direct binding of small compounds (such as drugs) to the TCR also implies that the TCR itself may constitute a potential drug target. The underlying assumption for this—namely, the wealth of pharmacological agents acting on many other classes of receptors that often have peptides or proteins as their “real,” endogenous ligands—has already been touched upon in *The P-I Concept: The TCR as the Antigen Binding Molecule?* It seems possible, albeit probably not feasible at this time, that the “arsenal” of modern drug technology could be used to find small molecules that block or enhance a particular, antigen-specific response of clinical relevance.

NOVEL GENETIC FACTORS LINKED TO DRUG HYPERSENSITIVITY

The idiosyncratic nature of hypersensitivity reactions has prompted an intensive search for genetic factors explaining their occurrence in only a small subset of treated persons.³⁰ In accordance with the (pro)hapten concept, the major emphasis was put on pharmacogenetic factors such as an altered metabolism, as the generation of a more reactive intermediate, able to modify autologous proteins, would have been the most stringent explanation for the occurrence of immunological side effects. However, associations of hypersensitivities with particular pharmacological genotypes remained often tenuous and even controversial,³¹ such as the slow acetylator phenotype reported to enhance the occurrence of side effects to SMX,³² and the moderate association of certain TNF- α promoter polymorphisms with carbamazepine hypersensitivity.³³

More recent studies focusing on immunological rather than metabolic factors have now revealed surprisingly clear associations of certain drug hypersensitivity reactions with HLA-class I alleles. In ~5% of treated patients, abacavir causes a severe hypersensitivity reaction affecting multiple organs. The majority of these patients with drug hypersensitivity carried the HLA-B*5701 allele. This association was strongest in Caucasians,³⁴ and the particular allele was present in 94.4% of patients but in only 1.7% of controls.³⁵ Possibly even more striking is the association of carbamazepine treatment with the appearance of Stevens-Johnson syndrome in Han-Chinese carrying the HLA-B 1502 allele.³⁶

This association is stronger than any other described so far for any HLA marker with a disease. In another case-control association study, the same authors identified HLA-B*5801 as an important genetic risk factor for severe allopurinol-induced cutaneous adverse reactions such as Stevens-Johnson syndrome and toxic epidermal necrolysis.³⁷

It is clear that such strong associations with HLA alleles support an important role for HLA molecules in drug hypersensitivity, and they certainly seem to favor the hapten concept at least for these drugs. However, although the association with HLA alleles is very strong, it is not a sufficient explanation: many patients with HLA-B*5801 are exposed to allopurinol, yet they do not develop hypersensitivity.³⁷ Caucasians do not show the association of HLA-B*5701 and carbamazepine hypersensitivity.^{34,35} As reported by the authors of these studies, other factors located in this region of chromosome 6 may be important as well (eg, hsp 70 and other genes). Most hypersensitivity reactions involve CD4+ T cells, which are MHC class II restricted.³ Last, not the HLA complex but the TCR, as its counterpart, might be crucial for the reaction, as the positive and negative selection of T cells in the thymus is codetermined by the autologous HLA molecules and peptides that can be presented, thus influencing the antigen repertoire of the individual patient.

CONCLUSIONS

Recent studies have shown surprisingly strong associations between particular MHC molecules and several drug-hypersensitivity reactions, lending further credence to the (pro)hapten concept. However, more and more in vitro studies imply that the (pro)hapten model as the sole molecular explanation for drug-induced hypersensitivity may not be sufficient, and that other possibilities should be considered. In fact, evidence is accumulating that certain drugs are able to activate T cells in ways that differ not only from the hapten model but also from other established concepts in immunology. It is becoming increasingly evident that not all drugs need to act as haptens. As far as drugs are concerned, it may be useful to draw inspiration from concepts of classical pharmacology, which have been established for greatly differing receptor classes. The potential reward may not only be a better understanding of drug-induced hypersensitivity reactions, but also novel means for immunomodulatory therapies.

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