

Haptens, prohaptens and prehaptens, or electrophiles and proelectrophiles

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It is argued that the term 'hapten', and derived terms such as 'pro-hapten' and 'pre-hapten' are ambiguous and unnecessary. It is proposed that their use be abandoned. Instead, when considering the chemical basis of skin sensitization, it is preferable to classify compounds according to the chemical reaction mechanisms by which they can modify proteins.

Key words: protein binding; chemical mechanism of action.

Low-molecular-weight chemicals (<1000 g/mol) that cause immunological responses, such as those leading to contact allergy, are often referred to in the dermatological literature as 'haptens'. This nomenclature is based on the concept in immunology that in order to sensitize the immune system specifically to a chemical, that chemical must covalently bind to (i.e. haptenate) protein to produce macromolecular antigens. It has also been recognized for sometime that certain low-molecular-weight chemicals are sensitizers but are not directly reactive towards proteins. To act by a covalent mode of action leading to haptenation, such chemicals require activation either via abiotic (biochemical) or metabolic (enzymatic) conversion to protein-reactive inter-

mediates or derivatives. Such compounds have been referred to generically as 'prohaptens' (1).

Lepoittevin has recently argued (2) that the nomenclature should be extended by addition of a new term, 'prehaptens'. The definitions proposed by Lepoittevin are

- Haptens – sensitizing chemicals that are directly protein reactive
- Prohaptens – sensitizing chemicals that are not protein reactive but are converted metabolically to protein-reactive derivatives
- Prehaptens – sensitizing chemicals that are not protein reactive but are converted abiotically to protein-reactive derivatives

The argument for defining new terms for the chemical classification of skin sensitizers is logical, and we agree with the motivation behind the proposal, which appears to be that the original 'prohapten' concept has tended to be interpreted as involving specific metabolic activation by enzymes, and that because specific metabolic activation is difficult to predict (due to a fundamental lack of knowledge of metabolism in the skin), this has impeded the development and acceptance of nonanimal methods for skin sensitization. Lepoittevin raises the question 'What is the proportion of non-protein-reactive sensitizers that need specific metabolic activation compared to those which are activated simply by air oxidation?' The implied answer (with which we would in principle agree, but in practice has not been demonstrated to date) is: very few. We do know that of all the sensitizing chemicals for which we have biological data, approximately two thirds of them are 'electrophiles' i.e. they possess chemical functionalities that enable them to covalently modify protein to form haptens. The other third of sensitizers are not directly protein reactive (i.e. they are not 'electrophiles'). Hence, to act by the classical 'hapten hypothesis', they must be activated by some means, on or in the skin. The logical conclusion from this analysis is that a potentially useful route to optimal nonanimal prediction of skin sensitization potential is by research into reaction chemistry.

In the emergence of a new era towards the development of alternative testing strategies for the prediction

of toxicological end-points including skin sensitization, we agree that there is a need to be more precise about the definitions we use to describe the different mechanisms by which sensitization may be explained. Although we agree with the need to correct any overemphasis on the role of specific metabolic activation of non-protein-reactive sensitizers, we are not convinced that the use of an extra nondescriptive term 'prehapten' is the best way to achieve this. We have 3 major concerns.

(1) The term 'hapten' has become ambiguous, being used with 2 meanings that are not compatible. Sometimes 'hapten' is used to denote the fragment bound to protein as a result of reaction between the protein and the sensitizing chemical and sometimes it is used to denote the sensitizing chemical itself. Sometimes both usages can be found in the same paper (including, we admit, some of our own). The distinction between the 2 meanings is not trivial. When a chemical reacts with a protein, its identity changes, in some cases very substantially. For example, when the sensitizer methyl dodecane sulphonate, $C_{12}H_{25}SO_3CH_3$, reacts with protein, only the CH_3 group (less than 6% by weight of the original molecule) becomes bound to the protein. According to the first usage, the 'hapten' is CH_3 , and according to the second usage, it is $C_{12}H_{25}SO_3CH_3$.

(2) It should be pointed out that not all metabolic processes are highly specific, and in many cases, it is not definitely known how the non-protein-reactive sensitizer is activated in skin. For example, how does the non-electrophile *p*-phenylene diamine (PPD) sensitize and how is the entity that becomes bound to protein derived? Is it via *p*-quinonediimine, via the trimeric oxidation product Bandrowski's base, via a Würster radical cation, or via a combination of many routes? Is the activation abiotic via abstraction of an electron by oxygen, or does it occur *in cutaneo* via 1-electron metabolic oxidation by a cytochrome P450 enzyme? What is the role of the observed PPD N-acetylation in the route to sensitization? We do not know. Similar questions can be raised for many other sensitizers, such as urushiol, the mixture of 3-alkyl and alkenyl catechols responsible for the sensitizing properties of poison ivy. Thus, in many cases, the information is simply

not available at present to discriminate between terms such as 'prohapten' and 'prehapten'. The important point, in our view, is to appreciate that activation of non-protein-reactive sensitizers can occur abiotically, by non-specific biochemical processes or (possibly, and if so probably only in rare cases) by specific metabolic processes. Also, that all such abiotic and metabolic conversion processes can occur simultaneously in the skin and even more so in *in vitro* cell-based systems and are difficult to tease apart analytically, as abiotic and metabolic activation can lead to the same electrophile.

(3) For practical purposes of hazard identification and risk assessment, there is a more important and fundamental distinction to be made for non-protein-reactive chemicals. These chemicals fall into 3 classes.

- (i) Non-sensitizers, e.g. *p*-aminobenzoic acid and lactic acid.

Not able to be activated to protein-reactive derivatives by any route

- (ii) Sensitizers (as pure chemical applied to skin), e.g. compounds such as PPD, hydroquinone, and 3-alkyl/alkenyl catechols that are not electrophilic as parent chemicals but sensitize when applied fresh to the skin. These compounds can be regarded as true sensitizers because they will inevitably be activated abiotically and/or metabolically in the timescale of any exposure situation or sensitization test.
- (iii) Chemicals causing skin sensitization by predictable impurities, e.g. compounds such as abietic acid, limonene, Δ^3 -carene, and other terpenes. The principal chemicals are not skin sensitizers; they can be obtained as pure chemicals that will not sensitize. However,

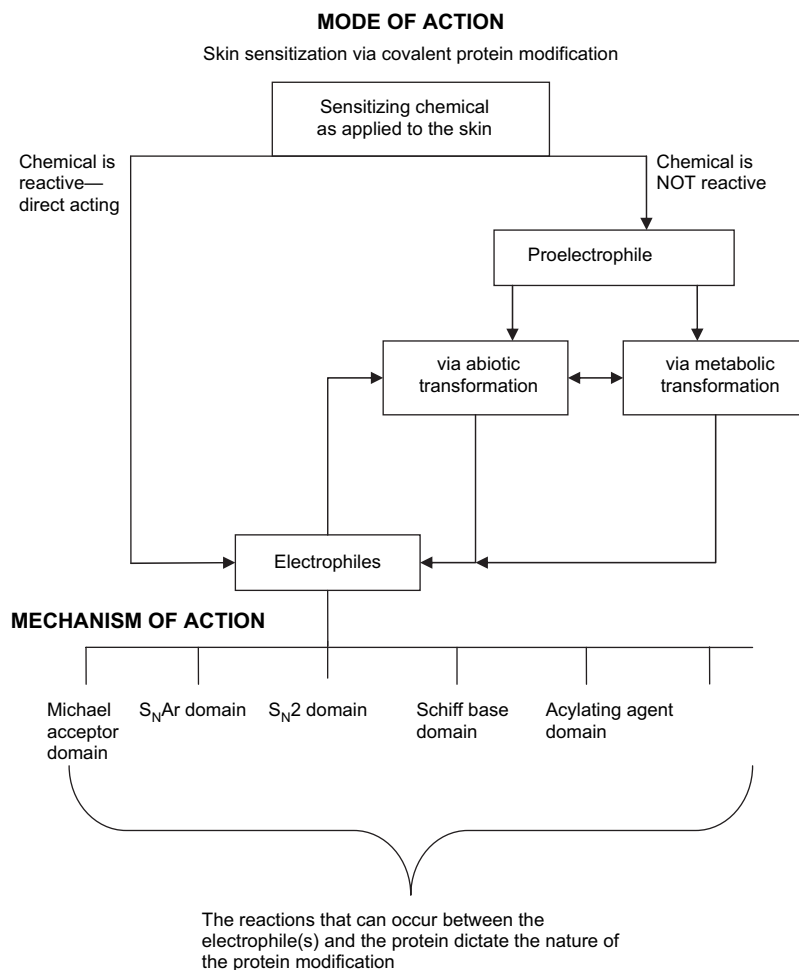


Fig. 1. Mechanism-based classification for skin sensitizers.

they can undergo deterioration (usually autooxidation by air) under certain storage conditions over a period of time, leading to the formation of sensitizing reactive impurities such as hydroperoxides.

Here, we propose an alternative and more precise nomenclature system for explaining the mechanisms of skin sensitization.

Although the term 'hapten' is strongly entrenched in the dermatological literature, in our view, its use with 2 different meanings makes it too imprecise for discussing the mechanisms by which chemicals cause skin sensitization. Furthermore, we see it as unnecessary, and in recent publications on skin sensitization, we have been able to dispense with the term completely. We therefore suggest that the use of the terms 'hapten', 'prohapten', and 'prehapten' be abandoned, as we intend to do in our future publications.

When considering the chemical basis of skin sensitization, we prefer to classify compounds according to their mechanisms of action, i.e. the chemical reaction mechanisms by which they can modify proteins (Fig. 1) (3,4). Skin sensitizing chemicals are either direct-acting electrophiles or not electrophiles, i.e. the latter can be designated as proelectrophiles (we include in this definition precursors of free radicals, whose 'philicity' is for single electrons) that require activation (abiotically and/or metabolically) to become electrophiles. The causative electrophiles in the process of sensitization can be subclassified into mechanistic domains according to the nature of the electrophilic reactions, e.g. Michael electrophiles (an example being benzoquinone), S_NAr electrophiles (an example being 2,4-dinitrochlorobenzene, DNCB). The proelectrophiles can be allocated to analogous mechanistic domains for the resulting electrophiles following activation (e.g. they can be pro-Michael electrophiles, an example being hydroquinone), without distinction as to whether the activation step to get to the electrophile is abiotic or metabolic.

The challenges in predicting sensitization potential from reaction chemistry may be summarized as follows. For pure chemicals that are

electrophiles, we can be confident that these chemicals have the potential to sensitize, and the challenge is determining the mechanisms by which they react with proteins, assessing if cross-reactivity can occur and quantifying the magnitude of the immune response. We note that having the potential to sensitize does not necessarily mean that a chemical will be a sensitizer. Its reactivity may be too low or it may be insufficiently hydrophobic. For the pure chemicals that are not electrophiles, there is the question whether or not they can act as proelectrophiles (i.e. activated to become sensitizing electrophiles) or whether they can be classified as 'nonreactive' and not sensitizing at all. At present, there remain some 'grey areas' where we cannot predict with confidence how nonelectrophiles will behave in this respect. Further experimental data are needed to help define better the mechanisms of action for the one third of chemicals that are known to be sensitizing proelectrophiles, i.e. are they converted abiotically or metabolically? Our suspicion is that transformation will result in electrophiles by a combination of routes and that proelectrophiles cannot be separated easily into distinct classes.

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series was supplemented with a textile dye mix and its separate constituents, our corticosteroid series with the corticosteroids available in the Swedish market, and some potentially cross-reacting corticosteroids. Besides alclometasone-17,21-dipropionate 1.0% dimethyl sulfoxide, -day (D)3, +D7, vehicle -D3, -D7 (present in the corticosteroid series), she reacted to nickel, cobalt, palladium, textile dye mix, disperse orange 3, mercaptomix, mercaptobenzothiazole, methylchloroisothiazolinone/methylisothiazolinone, and *Myroxylon perei*rae.

Case 2

A 54-year-old woman worked in a restaurant for several years when she started to get a hand dermatitis of unknown origin. She consulted her general practitioner and was prescribed with topical corticosteroid Locoid® cream, containing 0.1% Hc-17-B (Yamanouchi Pharma AB, Malmö, Sweden), but the disease was not investigated further. She used Locoid cream intermittently for 1 year. Despite this treatment, her hand eczema became worse, and because she suspected something at work, she was referred to us. She presented with numerous eczematous patches on several fingers (Fig. 1). She was patch tested to our standard series and materials from her work she suspected to be the cause of her dermatitis. At the first patch test reading on D3, no reactions were seen, but on D7, the second test reading, she presented with a reaction only to budesonide, 0.01% pet. She was then given a corticosteroid not cross-reacting with budesonide or Hc-17-B (viz. clobetasol propionate), and after 1 month, she

was free of eczema and has stayed free since, still working in the restaurant.

Discussion

The inclusion of corticosteroid markers into standard series has been advocated since the 1990s (4, 5). However, not all corticosteroid allergies are detected this way (1). Case 1 reacted to a corticosteroid, which is not present in our standard series and which is not detected with the 3 markers of corticosteroid allergy we have in our standard series, viz. tixocortol pivalate, budesonide, and Hc-17-B. When informed about her corticosteroid allergy, she remembered smarting pain and no improvement of treated eczematous skin after the use of Legederm® cream, containing 0.05% alclometasone-17,21-dipropionate (Schering-Plough AB, Stockholm, Sweden) in the 1980s. At that time, this had not been investigated further, and her doctor had just changed to another corticosteroid.

Case 2 was on the verge of changing her job when we saw her because she strongly suspected her work to be the cause of her chronic hand eczema. If a second late patch test reading had not been performed, she would have continued using the incriminating corticosteroid and her dermatitis would in all probability have remained even after changing jobs. Budesonide cross-reacts with Hc-17-B and is often positive when Hc-17-B is negative, even if the patient was primarily sensitized to Hc-17-B, which was the case here. Budesonide has not been present in any topical preparations in Sweden for over 10 years, and the patient denied that she had ever used the drug.

Case 1 is a good example of the need to patch test not only with the

marker molecules of corticosteroid allergy that should be present in any standard series but also with the corticosteroids used by the patient, either currently or previously. Such corticosteroids should be tested as pure substances in the appropriate vehicle and concentration (6). If positive, an extended corticosteroid series should be patch tested to detect cross-reacting corticosteroids and other contact allergies (7). As in case 1, the corticosteroid series could, of course, be patch tested at once, if the patient has a long-standing eczema and has used numerous corticosteroids.

Furthermore, both the cases are examples of the importance of reading corticosteroids not only after 3 or 4 days but also on a late occasion, i.e. after 7 days. In the first case, the contact allergy to alclometasone-17,21-dipropionate was of past relevance, but the drug could of course have been given to her once again, and in the second case, the outcome would have been very unfortunate.

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Fig. 1. Case 2. Numerous eczematous patches on several fingers at presentation.