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REVIEW ARTICLE

The human repeated insult patch test in the 21st century: A commentary

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Abstract

The human repeated insult patch test (HRIPT) is over half a century old, but is still used in several countries as a confirmatory test in the safety evaluation of skin sensitizers. This is despite the criticism it receives from an ethical perspective and regarding the scientific validity of such testing. In this commentary, the HRIPT is reviewed, with emphasis on ethical aspects and where the test can, and cannot, contribute in a scientifically meaningful manner to safety evaluation. It is concluded that where there is a specific rationale for testing, for example, to substantiate a no-effect level for a sensitizing chemical or to ensure that matrix effects are not making an unexpected contribution to sensitizing potency, then rigorous independent review may confirm that an HRIPT is ethical and scientifically justifiable. The possibility that sensitization may be induced in volunteers dictates that HRIPTs should be conducted rarely and in cases where the benefits overwhelmingly outweigh the risk. However, for the very large majority of HRIPTs conducted concerning the risk of skin sensitization, there is neither scientific justification nor any other merit.

Keywords: *Human repeated insult patch test; HRIPT; ethics; skin sensitization; risk assessment; thresholds; LLNA*

Introduction

For many years, the assessment of the risks to human health presented by skin-sensitizing chemicals was evaluated using an arcane approach: Where a hazardous substance was identified, its potency as a sensitizer was crudely estimated in one of a number of guinea pig assays and then, by comparative toxicology (i.e., comparing the new substance with others of similar potency) and/or by clinical studies, the potential risks to human health were judged. The inaccuracies and uncertainties inherent in this approach were relatively large and poorly appreciated, such that if considerable care was not exercised, outbreaks of allergic contact dermatitis (ACD) were likely (1,2).

In more recent years, a new approach to skin-sensitization potency assessment has been promulgated (3–5) and its application in quantitative risk assessment approach has been described (6–9). Through these means, some of the inaccuracies and uncertainties in risk assessment can be characterized and reduced. The approach predicts the skin-sensitization threshold in a human repeated insult patch test (HRIPT), but without the need to conduct such an assay, and then applies a number of considerations to it that lower the threshold, taking into account human variability, vehicle matrix, and exposure effects. In this way, an upper limit of acceptability is derived for an individual sensitizing substance in a particular exposure scenario (7,9). Although this approach does not normally require any human testing, in cases in which there are still uncertainties in the risk assessment, the conduct of appropriate experiments, including skin-permeation studies and/or an appropriately designed HRIPT, might well contribute substantially to the elimination of the uncertainties.

The question addressed in this commentary is whether and under what circumstances a human skin-sensitization assay can be carried out in a manner that is both scientifically robust and ethical, particularly given that one recent expert opinion concluded that, at least in relation to cosmetics and other consumer products, the HRIPT was judged unacceptable because of limited

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evidence of efficacy and concern that such studies were not ethical (10).

HRIPT: Protocol(s)

It is not appropriate in this commentary to give either a detailed protocol or the history of the HRIPT; such material can be found elsewhere (11–13). Typically, 9×24-hour or 48-hour exposures are delivered over a 3-week period to each of approximately 100 to 200 volunteers. After a 2-week break, a challenge exposure is made on both the induction site and a naïve site, again using a 24/48-hour patch. Skin reactions are scored over the subsequent few days. There are a number of minor technical variations to the protocol, including the type of patch, the use of occlusion or semiocclusion, the duration of the induction exposures, and the details of the grading scheme. Whereas there is no definitive method, it is widely acknowledged that the publication by Stotts provides the most thorough technical guide to the conduct and interpretation of an HRIPT (14). This has been supplemented in recent years by additional useful commentary (15,16).

HRIPT: Scientific considerations

Thresholds

Any clinical study involving human volunteers cannot be ethical unless it is scientifically sound (17). It was demonstrated many years ago that a properly conducted HRIPT was well capable of causing the induction of skin sensitization to a range of contact allergens, both strong and weaker ones (11–13). Thus, the protocol itself is not limiting in respect to its sensitivity. However, a number of scientific criticisms have been leveled at the HRIPT. The first and most common of these is that it is not possible to predict from approximately 100 healthy individuals what will occur in a large target/consumer population. This is quite correct; the statistical paper of Henderson and Riley is often appropriately quoted in this respect, indicating that a sensitization rate of < 1% is unlikely to be detected (18). It is for this reason that the simple conduct of an HRIPT with a finished formulation (be that a cosmetic, a household product, a pesticide formulation, or a topical medicine) is only very rarely of any scientific merit.

The second critical point is that an HRIPT deploys an exposure scenario (typically of 9×24-hour occluded exposures) that is wholly unlike any normal form of consumer or occupational exposure. This is also true. How is the circle to be squared? The answer to this conundrum is that whereas the HRIPT is in no way a tool to predict directly the frequency or intensity of effects in the normal exposed population, if undertaken appropriately, it is a tool that can permit 2 key elements of a risk assessment to be probed in detail in the species of concern. Firstly, where an HRIPT threshold has been predicted from other data, such as local lymph node assay (LLNA) or using a weight-of-evidence approach (19), then the human test can be done to confirm that it is indeed the upper limit of exposure for the HRIPT (which will not induce skin sensitization), not the limit for consumer exposure. Secondly, since it is well recognized that toxicology is not an exact science, the impact of vehicle matrix and of unanticipated chemical interactions in a product can be checked, normally to ensure that they are absent. These are discussed in more detail below.

Existing published data from the HRIPT permit the definition of skin-sensitization thresholds in that assay for a reasonable number of skin-sensitizing chemicals. Using this information, several different groups have reported the existence of a correlation between HRIPT and LLNA thresholds (the EC3 [concentration of test chemical required to provoke a 3-fold increase in lymph node cell proliferation] value) (5,9,19–23). The correlations reported are of course not perfect: The EC3 values are relatively robust, but the human data have been produced over time, to variable protocols, and are less so. In addition, although interspecies differences in hazard identification appear limited (24), it might be anticipated that there will be differences in the relative potency of some skin sensitizers between mice and humans. Thus, particularly where the risk assessment for an allergen suggests limited margin of error, one option to ensure the robustness of the assessment is to confirm the prediction of the human threshold by conduct of an HRIPT. This entails testing of concentration(s) of the chemicals at below threshold, rather than starting with the predicted threshold level. When subthreshold level has been demonstrated not to induce sensitization, then the investigator may choose to proceed to higher concentrations in a stepwise manner. With such a process, it is possible that skin sensitization to the chemical will be induced in 1 or 2 individuals. Clearly this would be the point where testing of any higher concentration is abandoned. In my experience, however, when an absence of effects at the predicted threshold is confirmed, testing at higher concentrations is not necessary.

Formulation effects

The second primary reason for conduct of an HRIPT is to try to confirm an absence of unexpected effects on a sensitizer incorporated into a formulation, such as the impact of the formulation matrix on epidermal bioavailability. The prediction of bioavailability in relation to skin sensitization is problematic, as a recent expert review

indicates (25). Furthermore, it is well recognized that vehicles can have an impact on the extent to which the inherent potency of a sensitizing substance is expressed (26,27). Although it may be possible to investigate some aspects of this using an *in vivo* method, such as the LLNA, where a risk assessment is more finely balanced, for example, between a clinical benefit and the potential induction of allergy to the medicament, then an HRIPT of the sensitizer in question in a proposed formulation may be considered necessary. In this way, any adverse impact of formulation/excipients on expressed sensitization potency could be disclosed. Using such a strategy would involve the generation of knowledge by standard toxicology testing on the substance such that its HRIPT threshold in a standard test vehicle was already known (19) and that an acceptable consumer exposure level has been determined by conduct of an exposure-based risk assessment. An HRIPT program involving testing below this threshold in potential formulations would serve to confirm whether there was any adverse impact on expressed potency.

In this situation, where the HRIPT outcome is negative, it does not mean that sensitization will not occur in the exposed population (see above), since the assay does not have the power to predict population effects. If, in contrast, the test shows evidence of the induction of skin sensitization, then it provides an indication that there is a problem with the risk assessment, thus allowing the safety evaluator a chance to reconsider before a product is placed on the market, potentially for uncontrolled use by millions of consumers.

It is worth mentioning here that in some countries, regulations governing the introduction of certain types of chemicals/products into the marketplace often require (although never explicitly) a human sensitization test to be undertaken. Where such testing represents a final decision point to determine whether a product is safe for the intended market, such a decision is likely to be flawed, largely because the test cannot predict an absence of effect in widespread consumer use (see above). It is not appropriate to go into greater detail here other than to say it is my view that such testing should not be the norm and should be considered only on a case-by-case basis after a rigorous assessment of scientific need and with the benefit of fully independent ethical review.

Hypoallergenicity

Finally, a comment is appropriate on the HRIPT and its use in "hypoallergenicity" assessment. The prefix "hypo-" means lower, less, and, therefore, *hypoallergenic* in a strict definition must mean less allergenic. The expression is relative and so requires a point of comparison. Something may be proven to be hypoallergenic in an HRIPT, even where the result is entirely negative, only where there is a reference product that has been shown to be positive. Of course, such reference data will be historic, as to knowingly test something already proven to be positive in an HRIPT would not be ethical. Which nicely brings us to more general ethical considerations.

HRIPT: Ethical considerations

It has been mentioned already that conduct of an HRIPT brings the risk of the induction of skin sensitization, and indeed, both uses of the HRIPT mentioned above cannot be entirely free of all risk of induction of skin sensitization. Whereas this risk clearly must be reduced to an absolute minimum, if the risk were zero, then the study would be scientifically flawed and consequently unethical. In reality, all human studies must involve some essential elements: that they are scientifically sound, that the risks to the panelists are understood and clearly explained, and that the individuals who participate have willingly give written fully informed consent and are free to withdraw at any time without giving a reason. Details pertaining to these matters have been fully discussed in relation to skin-irritation assessments of cosmetic ingredients and formulations (28–30). Critical also is that each study has been approved by a fully independent ethical review committee and takes place with appropriate medical supervision.

All the above is well and good, but the most common criticism of the HRIPT in relation to safety is that if it is done properly, then there must by definition be a risk, however small, that one or more of the participants may become sensitized. Clearly, and as mentioned above, this must be the case for both scientific and ethical reasons. However, for the study itself to benefit from independent ethical support, it is necessary not only to demonstrate that the risk to any individual is low, but also to understand what impact there may be on the health of any individual who does become sensitized. In contrast to skin irritation that may be induced in the cosmetic testing mentioned above, the induction of skin sensitization is assumed to be effectively lifelong. This important matter is discussed in some detail below.

Although the data are relatively limited, it does appear that all individuals are susceptible to the induction of skin sensitization—results of various studies with strong allergens such as p-phenylenediamine, 2,4-dinitrochlorobenzene, and potassium dichromate are consistent with this hypothesis (31–34). The frequency of contact allergy to allergens such as poison ivy and nickel in some populations is consistent with this argument (35,36). However there is undoubtedly a wide individual diversity of thresholds such that for the overwhelming majority of skin sensitizers, including fragrance, preservatives, and rubber allergens, only a small proportion

of those similarly exposed actually develop ACD. This is suggestive of an as-yet-uncharacterized particular susceptibility in certain individuals, a concept that I feel is already widely appreciated in a practical sense and that has been formally documented recently (37,38). What is key here is that there is an apparent subset of individuals who are more susceptible not only to the induction of skin sensitization, but also to the expression of eczema, as it is for the latter reason that they present for dermatologic investigation. The relative importance of increased susceptibility to induction versus elicitation in such individual is of course unknown. Furthermore, characterization of this issue is inevitably clouded by the fact that in many situations it is differences in exposure that dictate that individuals become sensitized as well as their particular degree of susceptibility.

How does the ACD patient compare with an individual who has developed skin sensitization in the context of an HRIPT? As indicated earlier, the HRIPT is not a predictor of general human population effects, but rather an investigation whose outcome is compared with that of historical controls (and thereby can be scientifically legitimized). Thus, it makes use of healthy human volunteers. As a consequence, it might be expected that individuals who do show evidence of skin sensitization at challenge are not necessarily identical to those represented by ACD patient groups. Support for this can be drawn from the experience that, to my knowledge, none of the limited number of individuals who were sensitized as a result of participation in an HRIPT have ever reported subsequent allergic eczema to their allergen. Furthermore, experimental studies have also demonstrated a failure to react to their skin sensitizer under normal exposure conditions (39,40). Finally, a prospective investigation by the dermatologist overseeing all HRIPTs in the Edinburgh area and who also runs the only patch test clinic in that area has indicated that none of his 5,000+ clinical ACD cases have arisen from HRIPT-related induction of sensitization (Dr. R. Aldridge, personal communication, 2006).

Summary

The HRIPT is not a predictive test to be used lightly. Where a safety assessment cannot be satisfactorily concluded and where there is a very clear preponderance of benefit over risk, then the conduct of an HRIPT may be ethically and scientifically justifiable. A rigorous safety and independent ethical review is required that, among other things, will demonstrate how data from the HRIPT would be integral to (and never be a replacement for) the risk assessment. The author remains concerned that for many HRIPTs currently conducted for the generation of reassurance regarding the risk of skin sensitization, there is neither scientific justification nor any other merit.

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