Skin Sensitization: Modeling the Human Adverse Response

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Summary

Assuring consumer safety without animal testing is a considerable challenge, but we remain confident it is ultimately achievable. In 2004, Unilever initiated a substantial research program to critically evaluate the feasibility of a new conceptual approach for consumer safety risk assessment (Fentem et al., 2008). Here we demonstrate significant progress in developing a non-animal risk assessment approach for skin sensitization. Previously, in collaboration with Entelos Inc., we developed a computational model of skin sensitization using the published literature (Maxwell and MacKay, 2008). Guided by this work, we now are developing a mechanistic model of skin sensitization that aims to interpret non-animal datasets, e.g., skin diffusion (Davies et al., 2011) and peptide reactivity (Aleksic et al., 2009) in the context of the known biology to predict the human sensitizer-specific T cell response, thereby allowing risk assessment decision making without new animal test data.

Keywords: animal alternatives, mathematical modeling, replacement, risk assessment, skin sensitization

1 Introduction

Unilever initiated a long-term research and capability development program in 2004 to evaluate and implement a new conceptual approach for assuring consumer safety without animal testing (Fentem et al., 2004, Westmoreland et al., 2010). Unilever's approach for safety assessment is risk-based, meaning that all available data on a new ingredient (including predicted levels of consumer exposure during product use) are used to assess the level of risk posed by its proposed use. The scientific challenge we continue to investigate is how novel non-animal datasets (i.e., data generated using *in chemico* and *in vitro* test methods) can be applied to risk assessment decision making to replace the need for new animal test data. Within our Skin Sensitization program we have made a long-term investment to define and explore the key toxicity pathways that drive the disease process (i.e., Allergic Contact Dermatitis) in humans, as we believe this mechanistic understanding should form a sound basis for our non-animal risk assessment approach (Maxwell et al., 2008). Here we share our progress in developing a mechanistically-based mathematical model of the induction of skin sensitization in humans.

2 Current risk assessment approach for skin sensitization

Decisions regarding the safety of consumer products (such as soaps, body lotions, and toothpaste) are made on the basis of a risk assessment (RA), in which data on the potential hazards of the ingredients in those products are interpreted in the context of the likely exposure to the product. To ensure that novel ingredients in our products do not induce skin sensitization (and hence do not cause Allergic Contact Dermatitis), our current RA approach interprets hazard characterization data in the context of the consumer exposure scenario (e.g., concentration of the ingredient in the product, how the product is used by consumers) to assess the risk to humans and thereby inform the safety decision (Felter et al., 2003). The mouse Local Lymph Node Assay (LLNA) is an accepted animal test method that, in addition to identifying sensitization potential, can be used to establish a dose-response relationship and assign a sensitizer potency class, from "weak" to "extreme" (Basketter et al., 2002). LLNA data can be used to derive a No Observed Adverse Effect Level (NOAEL) in humans that can be interpreted using a Quantitative Risk Assessment (QRA) approach to derive a theoretical safe level of exposure to the ingredient (Basketter et al., 2005; Felter et al., 2003). This theoretical safe level of exposure then can be interpreted (along with any other existing relevant clinical data) in the context of the predicted consumer exposure to reach an overall risk-based safety decision.

3 Non-animal risk assessment approach for skin sensitization

The immunobiology of skin sensitization has been investigated for many years and, consequently, is relatively well understood (Vandebriel and van Loveren, 2010). Our current mechanistic understanding is that, in order to induce a sensitizer-specific

T cell response a chemical must be able to penetrate the skin, bind covalently to skin proteins (termed haptenation), and induce sufficient skin inflammation to activate skin-resident dendritic cells (DCs), thereby ensuring that the haptenated proteins are taken up by activated DCs and transported to the draining lymph nodes (dLN) where they are processed and presented to hapten-specific T cells. T cell activation is dependent upon the successful binding of the T cell receptor (TCR) to a complementary hapten/peptide-MHC complex displayed on the DCs, followed by the induction of receptor and cytokine-mediated co-stimulatory signals. If all of these signals are appropriately delivered, then T cell proliferation will occur, resulting in an increase of the frequency of hapten-specific T cells. Upon reaching an unknown threshold number of hapten/peptide-specific T cells, an individual will be said to be sensitized and will experience an inflammatory response upon re-exposure to the sensitizer. These eczematous skin reactions (termed ACD) are driven by hapten-specific $CD4^+$ T-helper 1 (Th1) and $CD8^+$ cytotoxic T cells, and they increase in severity upon each subsequent re-exposure.

To test and explore the relative contributions of individual biological pathways thought to be key to the induction of skin sensitization, we previously developed an *in silico* mathematical model (termed the Skin Sensitization PhysioLab® (SSP) platform (Maxwell and MacKay, 2008)), in collaboration with Entelos® Inc. The SSP platform aimed to capture our current understanding of the pathways involved in the induction of skin sensitization, using the published literature to define and calibrate the biological relationships. Model development required extensive quantitative data analysis at the level of individual pathways, as well as whole-scale system dynamics. Calibration experiments were performed to ensure that the key biological mechanisms and dynamics of individual pathways were consistent with the published data. Furthermore, we conducted a sensitivity analysis on the model to determine the relative contributions of individual pathways to the induction of skin sensitization by measuring the impact of several pathways on hapten-specific T cell proliferation, using prototypical weak, moderate, and strong sensitizers. This analysis allowed us to evaluate the relative predictive power of different non-animal test methods and has subsequently aided the development of a mechanistic rationale for the interpretation of non-animal datasets.

4 New metrics for skin sensitization

Mathematical models have been a key tool in construction of the physiological based pharmacokinetic models applied in toxicology over the last four decades (Thompson et al., 2008). To predict whether skin sensitization will be induced following a given consumer exposure to a novel ingredient we have focused on developing two linked mathematical models: a model of "total modified skin protein" and a "T cell response" model. We have selected model inputs and outputs (or metrics of skin sensitization induction) based upon our evaluation of the SSP platform (Maxwell and MacKay, 2008). We are aware, however, that some key gaps remain in our understanding of the induction of skin sensitization in humans and, consequently, the definition of each model's structure also will be supported by a research program addressing these gaps.

5 "Total modified skin protein" model

As outlined above, we currently are developing a mathematical model in order to predict the total amount of modified skin protein that would be generated following a given consumer exposure to a given novel chemical. Such a model output can, in turn, be used as an input to our T cell response model (discussed below). Definition of the model structure requires experimental data to define the underlying biology (such as tissue volumes) and how the key toxicity pathways interrelate. Furthermore, to predict the "total modified skin protein" metric, the model requires chemical-specific input data on absorption and distribution of chemical within the skin, covalent modification of skin protein nucleophiles, and metabolic activation and/or clearance of the parent ingredient (see Fig. 1). Our current research into the quantification of each of these toxicity pathways is discussed below.

In order to induce skin sensitization a chemical must first gain access to the viable layers of the skin, with the most likely target site being the viable epidermis where immature DCs (termed Langerhans' cells; LCs) reside. The amount of chemical available and the duration of exposure to the epidermis depend on the rate of penetration, the distribution profile within the epidermis, and the removal of the chemical, either by metabolic or dermal capillary clearance. The epidermal bioavailability of the chemical can be summarized using kinetic models, yielding parameters such as the C_{max} (the maximum concentration in the epidermis), t_{max} (the time at which C_{max} occurs), and AUC (the area under the curve, a composite measure of the extent and duration of exposure). An experimental approach based upon *ex vivo* human skin recently has been investigated, whereby such parameters can be estimated for the epidermis and dermis following topical exposure (Davies et al., 2011). These experiments examined the effects of physiochemical parameters such as lipophilicity, volatility, and vehicle on the chemical kinetics of skin bioavailability.

The bioavailability of free chemical in the skin tissue also is influenced by skin metabolism, tissue absorption/binding, and clearance mechanisms. A previous literature review (Gibbs et al., 2007) revealed a lack of fundamental knowledge about human skin metabolism. However, the *in vitro* and *ex vivo* characterization of skin metabolism is challenging due to the loss of metabolic function almost immediately following biopsy (Oesch et al., 2007; Svensson, 2009). Nevertheless, if experimental methods could be developed that measure the metabolic clearance (or activation) of skin sensitizers, this information could be combined with data on penetration kinetics and protein reactivity to determine the amount of total modified protein in the skin. For the purposes of measuring bioavailability in skin,

it may also be important to develop experimental methods to determine intra- and extracellular concentrations of a chemical (or metabolite). The metabolic capacity of the skin is predominantly intracellular, so improved knowledge of the distribution of chemicals within the skin would help to refine the output from experimental measures of dermal bioavailability.

It is generally understood that any chemical (or a metabolite of it generated within the skin) must form a stable adduct with protein in the skin in order to stimulate an immune response (Landsteiner and Jacobs, 1935). Consequently, the stable modification of a protein by a reactive chemical (haptenation) is considered a key step in the induction of skin sensitization. We previously developed an *in chemico* peptide reactivity profiling assay that uses a panel of six single-nucleophile peptides (generic sequence AcFAAXAA, where X = Cys, Lys, Tyr, His or Arg, with H_2N-FAAAAA representing the N-terminal nucleophile), with the aim of determining the reactivity profile of a chemical with a high level of confidence (Aleksic et al., 2009). In addition to measurements of unchanged peptide and observation of the formed adduct(s), this approach permits the determination of reaction mechanisms (Aptula et al., 2007), specificity, and relative rates of reactions. The challenge ahead is to use a mathematical modeling approach to interpret these (or similar reactivity) data in the context of information on skin diffusion and metabolism to predict how much "total modified skin protein" would be generated following a given human skin exposure to a specified

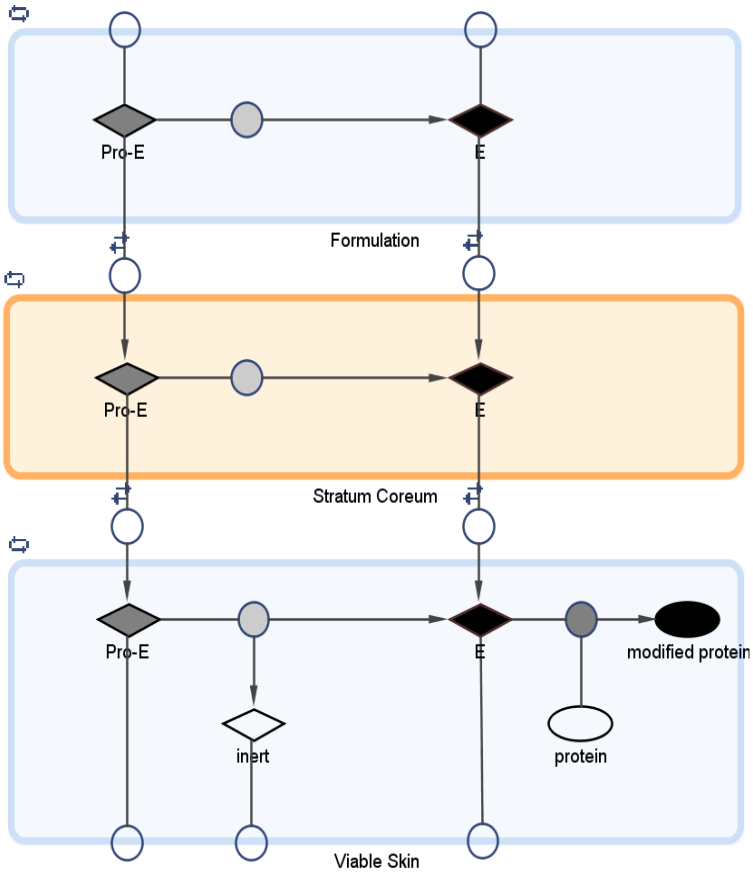

Fig. 1: A schematic of the proposed model structure required to predict the total amount of modified protein for a given consumer exposure

Model nodes represent quantities of interest and arrows represent processes.

Key shapes: diamond, xenobiotic; oval, proteins; transparent circle, clearance process (e.g., evaporation, capillary clearance, etc.); transparent circle with double arrows, diffusion; light grey circle, metabolism/transformation; dark grey circle, covalent binding

Key abbreviations: E, Electrophile/sensitizer; pro-E, pro-electrophile/sensitizer

sensitizer. Model development will be supported by research activities to help us better understand how inherent chemical reactivity induces skin inflammation and to deepen our knowledge of the metabolic pathways responsible for activation and/or detoxification of sensitizers by human skin.

6 T cell response model

In May 2010, we held a workshop (London, UK) to explore the relationship between sensitizer-induced T cell responses and sensitizer potency with experts from various disciplines, including immunology, mathematical modeling, and risk assessment (Kimber et al., manuscript in preparation). After two days of presentations and discussions the key conclusion was that, based on our current understanding, three theoretical metrics of the sensitizer-induced T cell response could be used to describe human sensitizer potency. These metrics were: the magnitude of the T cell response, particularly the vigor and duration of T cell proliferation, and the clonal expansion of sensitizer-specific T cells, as well as the quality of the T cell response, including the balance achieved between effector and regulatory T cells and, finally, the breadth of the T cell response, particularly the clonal diversity of the T cell response. Based on this workshop, we are developing a mathematical model, supported by a program of research, to establish which of these theoretical metrics (singular or combination) best describes human sensitizer potency and to what extent sensitizer-induced T cell responses differ fundamentally from protective T cell responses to known pathogens.

The initial objective for mathematical modeling of the T cell response is to predict the dynamics of the $CD8^+$ subset in humans following repeated exposure to a sensitizer, as this subset is thought to be the primary effector T cell population responsible for eliciting allergic contact dermatitis (Martin et al., 2010). When available, sensitizer-specific data will be used to build this model, while aspects of the model for which relevant sensitizer data are missing can be informed by knowledge of T cell dynamics from pathogen or vaccine responses (e.g., De Boer et al., 2001; Kaech and Ahmed, 2001). The predictions of "total modified skin protein" discussed above will be used to describe the timescale of immune system exposure to sensitizer antigen, which will drive the response. The numbers of $CD8^+$ T cells over time will be tracked from the activated lymph node to circulating in the blood and homing to skin. This separation of physiological compartments is novel for a T cell dynamics model, as they typically describe a single compartment (whether a lymph node (Maxwell and MacKay, 2008), spleen (Bocharov, 1998), or blood (Perelson and Nelson, 1999)). However, it is necessary in order to be able to test predictions for the blood and skin compartments where the relevant T cell numbers may be accessible for measurement. Naïve, cytotoxic, and memory $CD8^+$ T cells will be included in this model, and the output will represent the magnitude metric of the T cell response in skin sensitization.

Longer term modeling and research activities will aim to develop models of $CD4^+$ T cell subsets and responses to multiple epitopes with varying affinities to address the quality and breadth metrics. Furthermore, clinical research will be undertaken to benchmark "T cell response" model predictions against current clinical adverse/non-adverse thresholds for skin sensitization (e.g., measurable by diagnostic or human repeat-insult patch test; HRIPT). In so doing, we aim to ensure that the "T cell response" model can predict whether the human T cell response that would follow a given consumer exposure to a given sensitizer would be adverse or not (i.e., would the consumer become sensitized and thus potentially show a response upon re-exposure to the sensitizer).

7 Discussion

Assuring consumer safety without animal testing represents a formidable challenge. However, in recent years we have significantly increased our knowledge of the toxicity pathways driving the induction of skin sensitization. Consequently, we are developing a non-animal risk assessment approach for skin sensitization that aims to utilize our mechanistic understanding to integrate non-animal test method data to predict whether an adverse immune response could result from a given consumer exposure to a novel chemical. We are developing a mathematical model that can predict a "total modified skin protein" metric using chemical-specific non-animal test data. A "T cell response" model, also under development, will use "total modified skin protein" information to predict whether a sensitizer-induced T cell response will be induced and, if so, whether the response would be considered adverse (i.e., would the consumer be diagnosed as clinically sensitized). These mathematical models will be underpinned by research activities that will increase our understanding of key toxicity pathways and their impact on the adverse immune response. New non-animal test methods will be developed and existing test methods optimized to provide relevant information to inform model predictions for given chemicals and exposure scenarios. Furthermore, human-relevant *in vivo* benchmarks will be established through relevant clinical studies to replace our current dependence on sensitizer potency predictions derived from historical LLNA studies.

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