Applicability of GARD™skin for accurate assessment of challenging substances in the context of skin sensitization testing

Julia Li, Henrik Johansson, Andy Forreryd, Angelica Johansson - SenzaGen AB, Lund, Sweden

Conclusion

- GARDskin demonstrated an overall high applicability for the evaluated challenging substances with 80% predictive accuracy compared to existing human data.
- GARDskin demonstrated excellent applicability for pre/pro-haptens and low water solubility substances, correctly classifying all such compounds in the herein investigated dataset.
- GARDskin also showed high applicability for assessment of surfactants with 89% predictive accuracy compared to existing human data, correctly classifying 8 out of 9 internally tested surfactants, including well known challenging ones such as Sodium Dodecyl Sulphate (SDS) and Benzalkonium chloride.

Introduction

Current legislations and trends in predictive toxicology advocate a transition from *in vivo* methods for hazard and risk assessments to non-animal alternatives. However, certain groups of chemicals, including substances with severe membrane-damaging properties, pre- and pro-haptens, and those with high log P ratios, have been shown to be challenging to be accurately assessed using cell-based assays in the context of skin sensitization testing. The aim of this study was to evaluate the applicability of GARDskin for such challenging substances, using an overlapping subset of chemicals previously tested in an integrated tested strategy (ITS) based on validated, aqueous *in vitro* assays, as well as in a series of Reconstructed Human Epidermis (RHE)-based assays. [1]

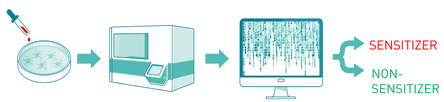

Figure 1. The general process of the GARDskin assay.

The GARDskin assay (Genomic Allergen Rapid Detection) is a robust *in vitro* assay for identification of potential chemical skin sensitizers with high performance and broad applicability. The assay evaluates the gene expression of endpoint-specific genomic biomarkers in a human dendritic-like cell line following exposure to the test substance. Exposure-induced gene expression patterns are analysed using pattern recognition and machine-learning technology, providing classifications of each test item as a skin sensitizer or a non-sensitizer. For a detailed description, see Johansson et al. 2019[2]. The assay has been recently subjected to a formal validation procedure (OECD TGP 4.106), with estimated reproducibility between laboratories of 92%, and the predictive accuracy of 94%.[2]

Materials and Methods

The applicability of GARDskin for a total of twelve challenging substances, including pre/pro-haptens, low water-soluble substances, two surfactants and three additional substances known to have conflictive results when comparing *in vitro* and *in vivo* data were evaluated in this study (Table 1). All twelve substances were selected from the Mehling et al. 2019 publication which reported results from three OECD validated *in vitro* methods, the "2 out of 3" Integrated Testing Strategy, three RHE-based models and the murine local lymph node assay (LLNA).[1] Human potency classification was available for ten out of the twelve substances.[7] The GARDskin prediction results were reported from previously published studies [4, 5], or from in house validation studies [3, 8]. Predictive accuracies were calculated by comparing skin sensitization classifications from different test methods to the available human data of each substance respectively. (N=10).

To further explore and substantiate the GARDskin applicability for surfactants, additional GARDskin data for a total of nine surfactants are presented in Table 2, in order to complement the Mehling dataset with respect to availability of human data.

References:
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Results and Discussion

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The GARDskin assay demonstrated an overall high applicability for the evaluated challenging substances, with 80% predictive accuracy compared to existing human data. GARDskin correctly classified all pre/pro-haptens and low water-soluble substances in the data set (Table 1). Furthermore, high applicability of GARDskin for severe membrane disruptive substances such as surfactants was demonstrated, with 89% predictive accuracy compared to existing human data (Table 2).

Two false positive results from GARDskin were obtained when comparing to the human data: Tween 80 (HP class 6) and Propyl paraben (HP class 5). Tween 80 is known to be consistently classified as a sensitizer in numerous *in vitro* assays, probably due to its severe cell membrane disrupting property. As for Propyl paraben, the positive result is most likely related to the ambiguous annotations for class 5 chemicals. Indeed, class 5 chemicals are appropriately considered as potential sensitizers, differentiated from true non-sensitizers of class 6 chemicals.

GARDskin is based on a dendritic-like cell line, expressing several metabolizing enzymes required for activation of pre/-pro haptens (e.g. ALDH, CYP, NAT-1, as verified by gene expression measurements. Data not shown). We hypothesize that the herein demonstrated GARDskin applicability can be ascribed to these cellular functions. Furthermore, the observed high predictive performance for substances with high Log P ratios is likely attributed to the high sensitivity of the assay in terms of required concentrations required to elicit a positive response. In addition, the solubility for hydrophobic substances is further increased using an extended panel of non-polar solvents compatible with the cellular system, as previously demonstrated.

Table 1. List of test substances and the prediction results from GARDskin in comparison with available *in vitro* and *in vivo* data.

Substance	#CAS	Why challenging?	DPRA[1]	KeratinoSens[1]	hCLAT[1]	2 out of 3[1]	SensCeeTox[1]	SENS-IS[1]	IL-18 epiCS[1]	Il-18 EpiDerm[1]	GARDskin	LLNA[1]	Human [7]
Resorcinol	108-46-3	Pre/pro-hapten	NS	NS	S	NS	NS	S	S	S	S[5]	S	S (HP4)
Aniline	62-53-3	Pre/pro-hapten	NS	NS	S	NS	NS	S	S	S	S[5]	S	S (HP4)
Abetic acid	514-10-3	Pre-hapten, high logP	S	S	NS	S	S	S	S	S	S[5]	S	S (HP3)
Farnesol	4602-84-0	Pre-hapten, high logP	NS	S	S	S	NS	S	S	S	S[5]	S	S (HP3)
Amylcinnamyl alcohol	101-85-9	High logP	NS	NS	S	NS	NS	S	S	S	S[5]	NS	S (HP4)
Benzoyl peroxide	94-36-0	High logP	S	NS	NS	NS	S	S	NS	NS	S[3]	S	S (HP3)
Isopropyl myristate	110-27-0	High logP	NS	NS	S	NS	S	S	NS	NS	NS[4]	S	NS (HP5)
Tween 80	9005-65-6	Cell membrane disruptive	S	S	NS	S	S	NS	NS	NS	S[5]	NS	NS (HP6)
Hexaethylene glycol monododecyl ether	3055-96-7	Cell membrane disruptive	NS	NS	NS	NS	S	NS	S	S	S[6]	S	No data
2-Chloro-6-methyl-3-aminophenol	84540-50-1	Conflictive results *in vitro* vs. *in vivo*	S	S	S	S	NS	S	S	S	S[6]	NS	No data
2-Hydroxy-4-methoxy bezophenone	131-57-7	Conflictive results *in vitro* vs. *in vivo*	No data	S	S	S	S	NS	NS	NS	S[6]	NS	S (HP4)
Propyl paraben	94-13-3	Conflictive results *in vitro* vs. *in vivo*	NS	S	S	S	S	S	S	S	S[5]	NS	NS (HP5)
Predictive accuracy - compared with Human data (N=10*)			44%	40%	60%	40%	30%	70%	70%	70%	80%		

*For DPRA, N=9

Table 2. List of surfactants and GARDskin prediction results in comparison with available *in vivo* data.

Substance	#CAS	GARDskin	LLNA	Human
3-dimethylaminopropylamine	109-55-7	S[5]	S (moderate) [10]	S (HP2) [7]
Benzalkonium chloride	63449-41-2/8001-54-5	S[8]	S (strong) [10]	S (HPT)[11], (HP5) [7]
Diethanolamine	111-42-2	S[5]	S (weak) [10]	S (HPT)[12], (HP5) [7]
Glyceryl monothioglycolate	30618-84-9	S[5, 9]	S (moderate) [10]	S (HP3) [7]
Octanoic acid	124-07-2	NS[5]	NS[10]	NS (HP6) [7]
Propylene glycol	57-55-6	NS[2, 5]	NS[10]	NS (HP5) [7]
Sodium dodecyl sulphate (SDS)	151-21-3	NS[9]	S (moderate) [10]	NS (HP6) [7]
Triethanolamine	102-71-6	NS[9]	NS[10]	NS (HP5) [7]
Tween 80	9005-65-6	S[5]	NS[10]	NS (HP6) [7]
Predictive accuracy - compared with Human data (N=9)		89%		

Abbreviations: NS=non-sensitizer, S=sensitizer, HP=Human potency classification, HPT=Human patch test

Contact Information

Henrik Johansson PhD,

henrik.johansson@senzagen.com