Applicability of GARD™skin for Accurate Assessment of Challenging Substances in the Context of Skin Sensitization Testing

J. Schmidt[1], A. Forreryd[2], H. Johansson[2], J. Li[2], A. Johansson[2]
[1]SenzaGen, Inc., Raleigh, NC, USA, [2]SenzaGen AB, Lund, Sweden, 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 assess using cell-based assays in the context of skin sensitization testing (Figure 1A). 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]

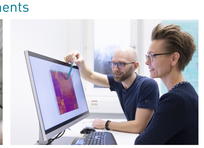
The GARDskin assay (Genomic Allergen Rapid Detection) is a robust *in vitro* assay for identification of potential chemical skin sensitizers with over 90% prediction accuracy and broad applicability. The assay is included in the OECD Test Guideline Program (OECD TGP 4.106) and has gone through a formal validation study.[2] 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 (Figure 1B).

A. Three types of "challenging substances" in the current OECD validated assays
- Pre- and Pro-haptens: requiring abiotic/biotic activation
- Hydrophobic substances: low solubility in cell media
- Surfactants: substances with cell membrane damaging properties

B. The GARDskin assay for skin sensitization hazard assessments

Step 1. Expose SenzaCells™ to the extracts at determined concentration.

Step 2. Measure the gene expression levels of the biomarkers, the genomic biomarker signature.



Step 3. GARD Data Analysis Application makes a binary prediction based on gene expression analysis.

Figure 1. Background: A. Types of "challenging substances" and B. GARDskin in 3 steps.

Materials and Methods

The applicability of GARDskin for a total of twelve challenging substances, including pre- and 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.

A. Study aim
- Evaluate the applicability of GARDskin for such challenging substances.
- Compare with ITS, RHE-based assays using the same dataset.

B. Method
- All 12 chemicals from (Mehling et al. 2019) were tested in GARDskin.
- Predictive performance evaluated against human data (available for 10 substances).
- GARDskin data were also generated for an additional dataset of 9 surfactants.

Figure 2. Study design: A. Study aim and B. Method

Results and Discussion

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-and 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. (*For DPRA, N=9)

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%		

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%		

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

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

References:
1. Mehling et al. The in vitro RHE skin sensitization assays: Applicability to challenging substances. Regulatory Toxicology and Pharmacology 2019
2. Johansson et al. Validation of the GARDskin assay for assessment of chemical skin sensitizers – ring trial results of predictive performance and reproducibility. Toxicological Sciences 2019
3. Johansson et al. Validation of the GARDpotency assay for assessment of chemical skin sensitizers 2018 (manuscript under review)
4. Forreryd et al. Predicting skin sensitizers with confidence - Using conformal prediction to determine applicability domain of GARD. Toxicology In Vitro 2018
5. Johansson et al. Evaluation of the GARD assay in a blind Cosmetic Europe study. ALTEX 2017
6. SenzaGen in-house data, not published
7. Basketter et al. Categorization of Chemicals According to Their Relative Human Skin Sensitizing Potency. Dermatitis. 2014
8. Forreryd et al., From genome-wide arrays to tailor-made biomarker readout – Progress towards routine analysis of skin sensitizing chemicals with GARD. 2016
9. Gradin et al. 2020 (Manuscript submitted)
10. Hoffmann et al. Non-animal methods to predict skin sensitization (I): the Cosmetics Europe database. 2018
11. Haneke et al. ICCVAM evaluation of the LLNA assay. 2001
12. Lessmann et al. Skin sensitizing properties of the ethanolamines mono-, di-, and triethanolamine. Data analysis of a multicentre surveillance network (IVDK) and review of the literature. 2009

Contacts

Henrik Johansson, PhD
henrik.johansson@senzagen.com

Andy Forreryd, PhD
Andy.Forreryd@senzagen.com

Joshua Schmidt, PhD
joshua.schmidt@senzagen.com