The GARD® Skin Assay: Investigation of the Applicability Domain of Indirectly Acting Haptens

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Conclusions

- The GARD®skin assay can accurately predict indirectly acting haptens and has the capacity to assess both pre- and pro-haptens as skin sensitizers.
- No increased risk of false negative classifications due to possible limitations in metabolic capacity of the cell system.

Introduction

Small immune-inducing compounds termed skin sensitizers, are known to cause allergic reactions in the skin, clinically manifested as Allergic Contact Dermatitis (ACD). Inherent properties of the substances make them react with skin proteins to form immune-inducing complexes. However, certain substances need to be transformed to protein-reactive intermediates, either biotically (pro-hapten) or abiotically (pre-hapten) before they can elicit an immune response.

Hazard assessments of skin sensitizers have traditionally been performed using animal experiments, but New Approach Methodologies (NAMs) are continually being used as replacements and are gaining regulatory acceptance. As the NAMs usually cover only a part of the necessary reactions needed to elicit an immune response and do not have the complexity of an *in vivo* system, it is crucial to determine the boundaries and applicability domains of the NAMs where they accurately can assess the investigated substances. One challenge with several NAMs is the capacity to have metabolic and chemical activity to accurately process pre- and pro-haptens, which can potentially produce false-negatives for such substances. Combining genomics and machine learning, the GARDskin assay [1] is a next-generation NAM, based on a human dendritic-like cell line, for hazard classification of skin sensitizers. Currently, the GARD assay is approaching regulatory acceptance as an OECD test guideline.

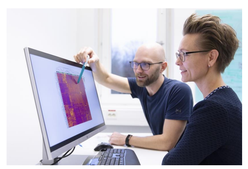
Here, we present a retrospective study on available GARDskin data in order to explore the applicability domain of the assay, specifically the capability to predict indirectly acting haptens.

Step 1

Expose Cells (the SenzaCell™ cell line) to the test sample at determined concentration.

Step 2

Measure the gene expression levels of 200 biomarkers, the genomic biomarker signature.



Step 3

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

Figure 1 The GARD®skin assay in three steps

Methods

Chemicals investigated in this study were mined from Corsini et al., 2021, Forreryd et al., 2016 and Johansson et al., 2017, 2019 [1,2,3,4] where the test chemicals were identified as indirectly acting haptens in the curated reference dataset [5] compiled by the OECD Expert Group for Defined Approaches for Skin Sensitization. Further subcategorization to either class, pro- or pre-haptens, were based on annotations from the mentioned study. In total, 25 substances were compiled from historical GARDskin testing. In short, GARDskin protocols used for cellular stimulation include exposure of compounds to a human dendritic-like cell line, SenzaCell™, followed by RNA purification and gene expression measurements. Gene levels are used as input values for the GARDskin classification algorithm, which is based on a Support Vector Machine prediction model. The output of the prediction model, the decision value (DV), gives the final classification as either a skin sensitizer if a positive DV (DV≥0) or as a non-sensitizer if it is a negative DV (DV≤0). The stepwise procedure for conducting the assay is illustrated in Figure 1.

Results

Results of individual hapten prediction in the GARDskin assay are shown in Table 1. Skin sensitizing hazard prediction for the GARDskin assay on indirectly acting haptens showed an overall sensitivity of 92.4% (23.1/25) and 87.5% (7/8) as compared to LLNA and human reference data, respectively. Further subcategorization into exclusively pre- (n=12) and pro-haptens (n=3), as well as 10 substances which could not unambiguously be assigned to a single class due to the duality of protein-reactivity, was made.

Out of the three exclusively acting pro-haptens, 67% (2/3) were correctly classified as skin sensitizers while all twelve pre-haptens were correctly classified as skin sensitizers. For the remainder of the chemicals (n=10), where no clear subcategorization could be made due to their reaction chemistry, 90% (9/10) were accurately classified as skin sensitizers.

Discussion

The aim of the present study was to investigate if the applicability domain of the GARDskin assay includes the ability to accurately predict indirectly acting haptens, compounds which require abiotic (pre-haptens) or biotic (pro-haptens) activation to gain peptide reactivity. With a sensitivity of 92.4% for indirectly acting haptens, this category of chemicals are deemed to be within the applicability domain of the GARDskin assay. However, as the metabolic capacity has currently not been fully characterized for the cell system used here, further subcategorization into pre- and pro-haptens was made. The sensitivity of 100% (pre-haptens) and 67% (pro-haptens), indicates that the SenzaCell™ cell line is capable of metabolically and chemically process indirectly acting haptens and the *in vitro* cell system is fit-for-purpose for such classes of chemicals.

In conclusion, the available GARDskin data shows that the SenzaCell™ cell line is able to chemically and/or metabolically activate pre- and pro-haptens and achieve accurate results on these compounds using the GARDskin assay.

References

[1] Johansson et al. 2019, Toxicological Sciences, 170 (2), p 374–38

[2] Corsini et al. 2021, EURL ECVAM - EU Reference Laboratory for alternatives to animal testing. GARDskin Assay Protocol

[3] Forreryd et al. 2016, Toxicology In Vitro, 37, p177-88

[4] Johansson et al. 2017, ALTEX, 34 (4), p 515-523

[5] OECD 2021, Series on Testing and Assessment 365 No. 336: Annex 2 of the Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Skin 366 Sensitisation

Table 1 GARDskin assessment of indirectly acting haptens

Chemical	CAS nr.	Mechanistic domain	Reference classifications		GARD weighted classification
			LLNA	Human	
2-Aminophenol	95-55-6	pre	Sens	NA	1
2-Nitro-p-phenylenediamine	5307-14-2	pre/pro	Sens	NA	1
2,5-Diaminotoluene sulfate	615-50-9	pre	Sens	NA	1
3-(Dimethylamino)propylamine	109-55-7	pro	Sens	NA	1
3-Aminophenol	591-27-5	pro-MA	Sens	NA	1
4-Amino-m-cresol	2835-99-6	pre/pro-MA	Sens	NA	1
Abietic acid	514-10-3	pre	Sens	NA	1
Aniline	62-53-3	pre/pro	Sens	Sens	0
Bromothalonil	35691-65-7	pre	Sens	NA	1
Chlorpromazine	50-53-3	pre/pro	Sens	Sens	1
Cinnamic alcohol	104-54-1	pre/pro	Sens	Sens	1
D-Limonene	5989-27-5	pre	Sens	NA	1
Dihydroeugenol	2785-87-7	pre/pro-MA	Sens	NA	1
Ethylene diamine (free base)	107-15-3	pro	Sens	NA	0.1[a]
Eugenol	97-53-0	pre/pro	Sens	Sens	1
Farnesol	4602-84-0	pre/pro	Sens	Sens	1
Geraniol	106-24-1	pre/pro	Sens	Sens	1
Hydroquinone	123-31-9	pre	Sens	NA	1
Isoeugenol	97-54-1	pre	Sens	Sens	1
Lauryl gallate	1166-52-5	pre	Sens	NA	1
Linalool	78-70-6	pre	Sens	NA	1
Metol	55-55-0	pre/pro	Sens	NA	1
p-Phenylenediamine	106-50-3	pre	Sens	Sens	1
Propyl gallate	121-79-9	pre	Sens	NA	1
Resorcinol	108-46-3	pre	Sens	NA	1

[a] Misclassified in 9/10 studies

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