GARD®skin Dose-Response for Photosensitization: Assessment of Reference Photoirritants and Photoallergens

Tim Lindberg[1], Gretchen Ritacco[2], Anders Jerre[1], Robin Gradin[1], Andy Forreryd[1], Henrik Johansson[1], Anne Marie Api[2]
[1]SenzaGen, Lund, Sweden, [2]Research Institute for Fragrance Materials. Woodcliff lake, NJ, USA

Abstract ID#4456

1. Introduction

Chemicals of different categories, such as cosmetics and drugs, have the potential to become photoactivated when exposed to UV-light, giving rise to otherwise dormant adverse effects, commonly referred to as phototoxicity. Such chemicals may be further categorized as photoallergens and photoirritants. While photoallergens give rise to a Type IV delayed hypersensitivity through a process known as sensitization, typically manifested as allergic contact dermatitis, photoirritants typically induce local and acute, albeit reversable, irritation at the site of exposure. In terms of risk management, the distinction of the two types of chemicals is important, as the hazardous exposure effects of photoirritants can be reduced by concentration limits, whereas photoallergenic properties currently results in a ban of the chemical at hand. Currently, no predictive method capable of accurately classifying and differentiating between the different types of phototoxicity exist.

Here, we demonstrate the ability of the GARD™skin Dose-Response method to predict phototoxic property of test chemicals and differentiate between photoallergens and photoirritants, by incorporating a UV irradiation step in the otherwise standardized protocols for test chemical exposure of cell cultures (Figure 1).

Step 1. The SenzaCell cell line is utilized as an *in vitro* cell system mimicking dendritic cells.

Step 2. UV-light is administered in conjunction with cell exposure of test chemicals.

Step 3. Gene expression analysis is performed in order to evaluate a set of predictive genomic biomarkers.

Step 4. Gene expression data is analysed using the GARDskin prediction model, allowing for classification of sensitizers.

Readout: Decision Value (DV) > 0 = Sensitizer Decision Value (DV) < 0 = Non sensitizer

Figure 1. Workflow used for assessment and phototoxicity using GARDskin Dose-Response.

2. Methods

The GARDskin Dose-Response is a method for quantitative assessment of sensitizing potency, based on acquisition of GARDskin measurements in a titrated range of test chemical exposure concentrations. From generated dose-response relationships, a cDV0-value is derived, which corresponds to the least required dose able to generate a positive response in the GARDskin assay. Thus, the GARDskin Dose-Response may be viewed as an *in vitro* analogue to the LLNA (Figure 2).

In the present study, GARDskin Dose-Response protocols were further amended with a UV irradiation step in conjunction with cellular exposure to test chemicals (3.1 J/cm2). It was hypothesized that a test chemical exposed to UV-irradiation that exhibits a significant decrease in cDV0 (i.e., indicative of an increase in sensitizing potency), as compared to the non-irradiated counterpart, may be classified as a photoallergen. Furthermore, it was hypothesized that a test chemical exposed to UV-irradiation that exhibits a significant decrease in cell viability (i.e., indicative of an increase in cytotoxicity), as compared to the non-irradiated counterpart, may be classified predominantly as a photoirritant. The proposed protocols and classification scheme were evaluated by assessment of 12 test chemicals, of which 6 were considered photoallergens and photoirritants, respectively, based on clinical and *in vivo* reference data.

	GARD	LLNA
Response value	DV	SI
Binary Threshold	DV = 0	SI = 3
Readout	cDV_0 (DV_0 Concentration)	EC3 Concentration

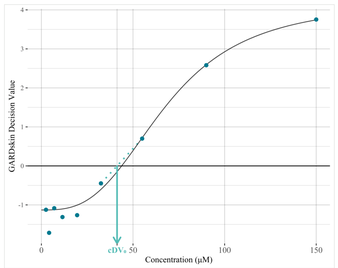

Figure 2. The GARDskin Dose-Response method is an *in vitro* analogue to the LLNA.

3. Results

The 12 test chemicals were evaluated in the GARDskin Dose-Response method in order to estimate their sensitizing potency, with and without the administration of UV irradiation. As an illustrative example, consider test chemicals dichlorophene and anthracene (Figure 3).

Here, dichlorophene is considered a conventional sensitizer, due to an established cDV0-value in the absence of UV irradiation. However, the sensitizing potency is increased, as monitored by a decrease in the cDV0-value, following UV irradiation. Lastly, the cytotoxic properties of the test chemical, as monitored and adjusted for by the top GARD input concentration in the titration range, remain unchanged following UV irradiation.

Thus, dichlorophene was classified as a photoallergen. Considering anthracene, it was considered a conventional non-sensitizer in the absence of UV irradiation. Following UV irradiation, anthracene becomes photoactivated and is now appropriately considered a relatively potent sensitizer, as derived from the cDV0-value. However, UV irradiation has also affected the cytotoxic properties, as monitored by the significant decrease of the top GARD input concentration used in the titration range. Thus, anthracene was classified as predominantly a photoirritant. Results from all test chemicals are summarized in Table 1.

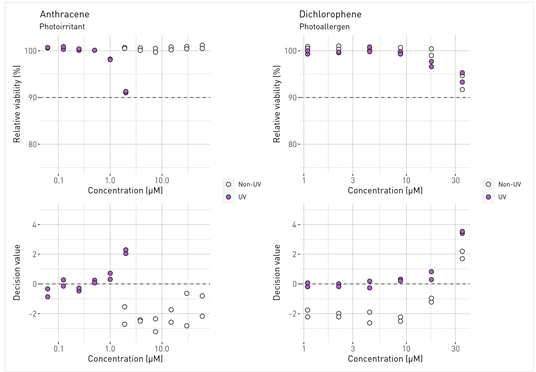

Figure 3. An illustrative example of the classification scheme used to differentially assess photoirritants and photoallergens.
Top figures: Cytotoxicity assessment.
Bottom Figures: GARDskin Dose-Response cDV0 prediction

4. Discussion

We propose a novel testing strategy for assessment of phototoxicity, based on the GARDskin Dose-Response method, amended with an experimental procedure for administration of UV irradiation in conjunction with test chemical exposure. A tentative classification scheme correctly classified 5/6 photoirritants and 3/6 photoallergens, as compared to reference values derived from clinical and *in vivo* data.

Moving forward, the tentative classification scheme will benefit from rigorous heuristics based on classification thresholds. Based on the herein reported data, a twofold increase in sensitizing potency of UV irradiated test chemicals, as compared to the non-irradiated counterpart, seems to be indicative of photoactivation and induced phototoxicity. Additional data from a larger set of test chemicals may be warranted to increase the confidence in such observations, which would enable the finalization of a frozen prediction model. In addition, it must be emphasized that the herein proposed tentative classification has been guided by available reference data, which was used to divide class labels by their mode of phototoxicity. Whether or not it is feasible to assume that any one test chemical may exhibit both modes of phototoxicity upon human exposure must be further elucidated, in order to construct the best possible classification scheme in a finalized prediction model.

In conclusion, the herein presented data demonstrates the ability of GARDskin Dose-Response, in combination with an experimental procedure for administration of UV irradiation, to predict and differentiate between photoallergens and photoirritants, a toxicological endpoint for which standardized and accepted methods currently do not exist.

Table 1. Summarized results non-UV vs UV treated samples

Reference Material		cDV_0 non-UV (µM)	Highest Input Conc. non-UV (µM)	cDV_0 UV (µM)	Highest Input Conc. UV (µM)	Ratio cDV_0 (non-UV/UV)	Ratio Input Conc. (non-UV/UV)	Prediction
Dimethyl anthranilate	PHOTO IRRITANTS	461	500	32.5	60	14.2	8.3	Photoirritant
Methyl B-Naphthyl Ketone		279	450	23.6	125	11.82	3.6	Photoirritant
Anthracene		Non-Sensitizer	60	0.232	2	Sensitizer	30	Photoirritant
Acridine		136	250	3.82	5	35.6	50	Photoirritant
Naproxen		429	500	124	500	3.46	1	Photoallergen
5-Methoxypsoralen		Non-Sensitizer	100	0.001	0.075	Sensitizer	1333	Photoirritant
7-Ethoxy-4-methylcoumarin	PHOTO ALLERGENS	121	350	7.2	300	16.8	1.17	Photoallergen
Dichlorophene		23.8	35	4.98	35	4.78	1	Photoallergen
Fenticlor		26.1	40	34.5	50	0.76	0.8	Non-Phototoxic
Hexachlorophene		8.16	20	7.9	20	1.03	1	Non-Phototoxic
Isoniazid		Non-Sensitizer	500	382	500	Sensitizer	1	Photoallergen
Musk Ambrette		28.8	125	22.8	30	1.26	4.2	Photoirritant

5. Conclusions

- Increase in cytotoxicity after UV exposure strongly linked with predominantly photo irritating properties.
- Decrease in GARDskin Dose-Response cDV0-value after UV exposure indicative of photoallergenic properties

Contact:
Tim Lindberg, PhD,
tim.lindberg@senzagen.com