Identification of skin sensitizers in natural mixtures

Key words: hair dyes, mixtures, cosmetics, chemical

Introduction

Henna is a natural dye traditionally used in several regions of the world. Henna dyes are extracted from the plant *Lawsonia inermis* with lawsone as the main natural pigment.

Nowadays, henna based hair colouring products are commercially available and are often considered as safer alternatives to synthetic hair dyes containing known skin sensitizers such as *p*-phenylenediamine (PPD). However, skin allergic reactions to henna products have also been reported.

A pilot study was performed to identify potential skin sensitizers in commercially available henna products using the GARD™skin assay.

The study was an international collaboration between the Department of Immunotechnology, Lund University in Sweden and the Laboratory of Research and Education in *In Vitro* Toxicology, Federal University of Goiás in Brazil. For more details, please see the original publication (de Ávila et al. Contact Dermatitis, 2019).

Assay Principle

The GARD™–Genomic Allergen Rapid Detection – platform is a state-of-the-art *in vitro* assay for identification of chemical sensitizers.

The GARD™skin assay is for identification of chemical skin sensitizers, with a predictive accuracy of around 90%.

Materials & Methods

Prior to the main study, ten commonly used hair dye ingredients (Table 1), including lawsone and PPD, were tested using GARD™ skin in order to confirm that the assay is applicable for hair dye ingredients in general.

A total of ten commercially available henna-based products (item 1 to 7 for hair and item 8 to 10 for eyebrow colouring) were purchased at local markets (Goiânia, GO, Brazil). All ten products were in the form of powder mixtures, claiming henna as the main ingredient. Nine of the products listed other natural and synthetic ingredients, of which two listed PPD. Please see Table 2 for the summary of labelled ingredients for each product.

The presence of lawsone and PPD was then examined using high performance liquid chromatography (HPLC). The detected levels (w/w%) are shown in Table 2.

In the end, all ten products were tested for skin sensitization hazard identification using the GARD™skin assay. For all the products water was used as solvent.

Figure 1. shows an overview process of the GARD™ assays. For the full procedures, see Johansson et al. ALTEX, 2017.

Figure 1. The general process of the GARD™ platform

The GARD™ platform is based on SenzaCells™, a human dendritic-like cell line. The assay protocol starts with identification of a suitable input concentration of the test item where the 90% SenzaCells™ survive. In the next step, a new batch of cells are exposed to the identified concentration and incubated for 24 hours. Total RNA is isolated and followed by gene expression profiling using NanoString technology. The gene expression pattern is analysed in the GARD™ data analysis application using a prediction model based on a machine learning algorithm. GARD™ skin gives a binary result: if the test item is a skin sensitizer or non-sensitizer.

Results & Discussions

The GARD™skin prediction for the selected hair dye ingredients showed a concordance of 73.3% in comparison to existing animal data and 100% when compared to existing human data (Table 1). The result indicates a high prediction performance of the assay for identification of skin sensitizers in hair dye ingredients.

It is notable that the natural key ingredient for henna dyes, lawsone, was predicted as a skin sensitizer by the GARD™skin assay. As a comparison to the result, the existing animal data is considered inconclusive and human data is not available.

When it comes to the evaluation of the commercially available henna products, all tested mixtures were predicted as sensitizers in the GARD™skin assay (Table 2).

As PPD was detected in all 10 tested products (even though only 2 of them were listed in the ingredient labels), it is difficult to draw a conclusion on whether the skin sensitization potential comes from lawsone or PPD alone or from the mixtures as combined effects.

Conclusions

This pilot study demonstrated the applicability of the GARD™skin assay for identification of skin sensitizers in hair dye ingredients, delivering high prediction performance, consistent with existing human data.

The study also indicated that GARD™skin is a promising *in vitro* model to identify skin sensitizers in natural mixtures.

Reference

de Ávila *et al.* Evaluation of *in vitro* testing strategies for hazard assessment of the skin sensitization potential of "real-life" mixtures: the case of henna-based hair-colouring products containing *p*-phenylenediamine. Contact Dermatitis, 2019.

Johansson *et al.* Evaluation of the GARD™ assay in a blind Cosmetics Europe study. ALTEX, 2017.

Table 1. GARD™skin prediction results for 6 selected reference controls and 10 hair dye ingredients.

Test items	Human data	Animal data	GARD™skin prediction
Reference controls			
Dimethyl sulfoxide	NS	NS	NS
Glycerol	NS	NS	NS
Sodium dodecyl sulfate	NS	S	NS
2,4-Dinitrochlorobenzene	S	S	S
Eugenol	S	S	S
2-Hydroxyethyl acrylate	S	S	S
Hair dye ingredients			
1,4-Diaminoanthraquinone	S	S	S
2-Amino-3-hydroxypyridine	S	NS	S
Lawsone	NA	Inconclusive	S
5-Amino-o-cresol	S	S	S
Hydroquinone	S	S	S
p-Phenylenediamine (PPD)	S	S	S
Resorcinol	S	S	S
Disperse orange 3	S	S	S
Basic red 51	NA	NS	S
Pyrogallol	S	NS	S

Table 2. For the 10 commercially available henna products: a summary of the product information including ingredients.

Test items	Ingredients declared on the label				Levels (w/w%)		GARDskin prediction
	Henna	Other natural	PPD	Other synthetic	Lawsone	PPD	
1	+				0.518 ± 0.003	1.091 ± 0.028	S
2	+	+	+	+	ND	2.970 ± 0.046	S
3	+	+		+	0.188 ± 0.001	0.030 ± 0.001	S
4	+	+		+	0.320 ± 0.002	0.032 ± 0.006	S
5	+	+			0.041 ± 0.001	4.321 ± 0.028	S
6	+	+			0.113 ± 0.001	1.020 ± 0.100	S
7	+	+		+	0.359 ± 0.001	0.577 ± 0.015	S
8	+	+	+	+	0.158 ± 0.001	2.541 ± 0.057	S
9	+	+			0.103 ± 0.001	0.760 ± 0.017	S
10	+	+			0.032 ± 0.001	3.354 ± 0.163	S

S: sensitizer, NS: non-sensitizer, ND: not detected, NA: data not available from the literatures

Table 1 and Table 2 are adapted from the original publication (de Ávila et al. Contact Dermatitis, 2019)

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