Potency Ranking of Dermal Sensitizing Chemicals Using the IVSA and epiCS® Skin Tissues

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ABSTRACT

Human 3D reconstructed skin epidermal equivalents have been shown to release IL-18 in response to a wide range of dermal sensitizing chemicals. The concentration of these chemicals that produce greater than a threshold positive response (Stimulation Index, SI ≥ 2.0) is correlated to their potency or strength in an ***In Vitro* Sensitization Assay (IVSA).** In our experiments, 4-Nitrobenzyl bromide (NBB) and 2,4-Dinitrochlorobenzene (DNCB) were strong inducers of IL-18 secretion into the culture medium (SI-2 = 0.02% and 0.03%, respectively). The strong sensitizer p-Phenylenediamine (PPD) had an SI-2 of 0.13%. Cinnamaldehyde (CA) (SI-2 = 0.33%) and Isoeugenol (IE) (SI-2 = 0.56%) were moderate sensitizers, while Eugenol (EU) (SI-2 = 0.75), Resorcinol (RES) (SI-2 = 2.9%) and Hexylcinnamaldehyde (HCA) (SI-2 = 8.08%) were weak sensitizers. Sensitizer potency ranked using an SI-2 as follows: NBB > DNCB > PPD > CA > IE > EU > RES > HCA, with NBB, DNCB and PPD classified as strong, CA, IE and EU as moderate, and RES and HCA classified as weak sensitizers. Of the total of 18 chemicals tested, seven were irritants and two were non-sensitizers (Glycerol and Isopropanol); of these, only Chlorobenzene (50%) was incorrectly predicted as a sensitizer. epiCS® gave an Accuracy of 89% and Sensitivity of 89%, and all other Cooper Statistics (Specificity, Negative and Positive Predictivity) values were 89%. In summary, measuring IL-18 release from 3D tissues allows for highly accurate and sensitive identification of dermal sensitizers. Also, the ability to rank-order potency of these chemicals based on SI-2.0 values of IL-18 secretion is a powerful tool for further classification into potency categories.

INTRODUCTION

The CDC estimates more than 13 million individuals in the US are likely exposed to chemicals that cause skin diseases. Many of these skin diseases are caused by contact dermatitis, a common type of illness associated with many occupational hazards, costing an estimated one billion dollars annually in healthcare and lost productivity (http://www.cdc.gov/niosh/topics/skin/). Currently there are no fully validated and regulatory accepted animal-alternative methods available to assess for Allergic Contact Dermatitis (ACD) / sensitization. In ACD, keratinocytes in the skin are the first to contact and elicit a response to allergens during an exposure. The 3D human epidermis equivalent epiCS® is reconstructed from normal human primary epidermal keratinocytes. Keratinocytes have been shown to secrete a wide range of cytokines. Evidence demonstrates that cytokine IL-18 is an essential component of dermal sensitization. Most notable it has been shown using IL-18-deficient mice that IL-18 is not required for irritation contact dermatitis, but is required for an optimal ACD response (*Antonopoulos et al., 2008. Journal of Leukocyte Biology 83: 361-7*). An IL-18 endpoint has been used to predict sensitization in tissue models using filter paper applications (*Gibbs et al., 2013, Toxicology and Applied Pharmacology 272(2):529-41*). To identify sensitizing compounds, we measured IL-18 secretion from epiCS® after treatment with pure chemicals via direct chemical application.

MATERIALS AND METHODS

Sensitizers and irritants/non-sensitizers were topically applied directly to the stratum corneum of epiCS® tissues. The primary vehicles used were Ethanol and Acetone:Olive Oil (AOO). At 24 hours post-chemical application, media was sampled and analyzed by ELISA (MBL, Nagano, Japan) for secreted IL-18. IL-18 responses were measured as a **Stimulation Index (SI)**, a fold increase above vehicle control. Tissues were washed and then tissue viability was measured by the MTT assay. The chemical concentration that results in 50% loss in tissue viability (**TV50**) was calculated when possible. At least two independent experiments were performed for each chemical. An SI of 2.0 was chosen as a cut-off for a positive response. A material was considered positive if at least one concentration of the material tested positive in at least two independent experiments.

RESULTS

Table 1. Contingency Table (SI=2 of IL-18)

	Known +	Known –	Total
Tested +	**8**	**1**	**9**
Tested –	**1**	**8**	**9**
Total	**9**	**9**	**18**

Accuracy	**89%**	**(16/18)**
Sensitivity	**89%**	**(8/9)**
Specificity	**89%**	**(8/9)**
Positive Predictivity	**89%**	**(8/9)**
Negative Predictivity	**89%**	**(8/9)**

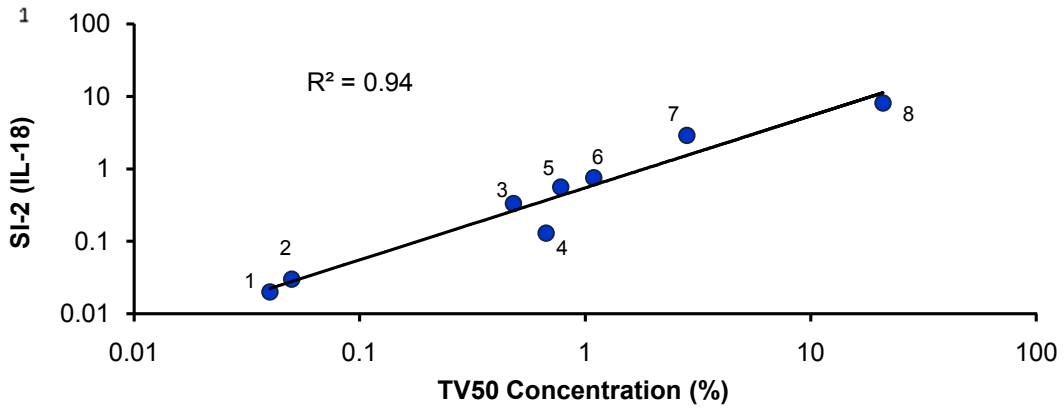
RESULTS (cont'd)

Table 2. Chemicals Tested in epiCS® IVSA

	Chemicals	IL-18[a] Result	SI-2[b] %	TV50[c]	LLNA[d] EC3 %	Human[e] DSA_{05} ($\mu g/cm^2$)
	Sensitizer					
1*	**4-Nitrobenzylbromide (NBB)**	+	0.02	0.04	0.05	—
2	**1-Chloro-2,4-Dinitrobenzene (DNCB)**	+	0.03	0.05	0.03	3.80
3	**p-Phenylenediamine (PPD)**	+	0.13	0.67	1.31	176
4	**Cinnamaldehyde**	+	0.33	0.48	1.71	634
5	**IsoEugenol**	+	0.56	0.78	1.71	1,054
6	**Eugenol**	+	0.75	1.09	11.73	5,926
7	**Resorcinol**	+	2.90	2.82	5.5	—
8	**α-HCA**	+	8.08	20.91	9.37	23,622
	Cinnamyl Alcohol (Cinn-OH)	–	na	1.40	21.0	13,747
	Irritants					
	Lactic Acid	–	na	3.11	na	na
	Phenol	–	na	2.22	na	na
	Sodium Dodecyl Sulfate (SDS)	–	na	—	na	na
	Methyl Salicylate	–	na	3.16	na	na
	Chlorobenzene	+	na	16.40	na	na
	Salicylic Acid	–	na	2.5	na	na
	Tween® 20	–	na	—	na	na
	Non-Toxic					
	Glycerol	–	na	—	na	na
	Isopropanol	–	na	—	na	na

[a]Using a SI-2 as the cutoff for a positive response with IL-18 in epiCS® tissues
[b]The chemical concentration (%) that achieved an SI-2 with IL-18 in epiCS® tissues
[c]The chemical concentration (%) that achieved 50% loss is tissue viability (TV50) in epiCS®
[d]LLNA EC3(%)
[e]Human DSA_{05} ($\mu g/cm^2$) data as reported in *Gibbs et al., 2013, Toxicology and Applied Pharmacology 272(2):529-41*
na = not applicable
*see Figure 1

Figure 1. Correlation of TV50 (EC50) and IL-18 SI-2



RESULTS (cont'd)

Figure 2a. Irritants in the IVSA

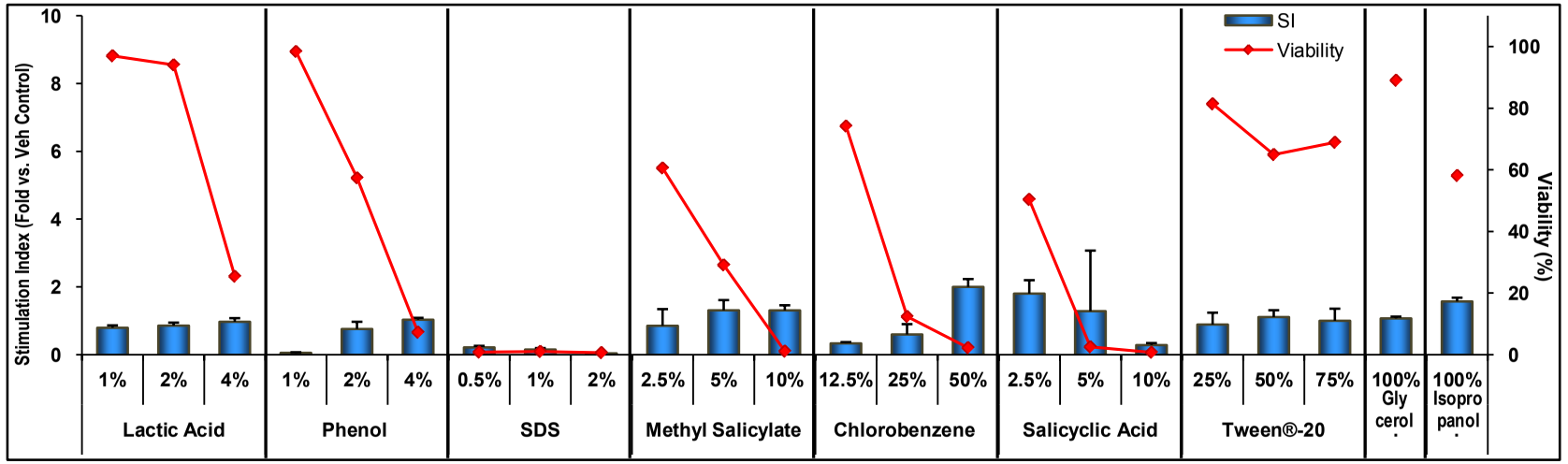
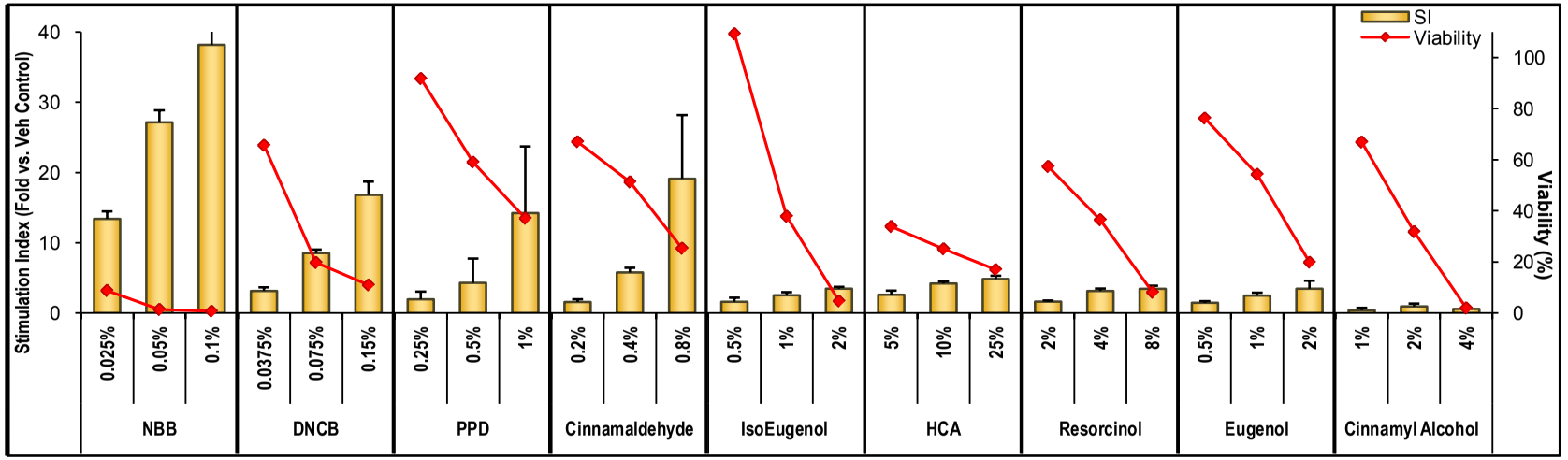
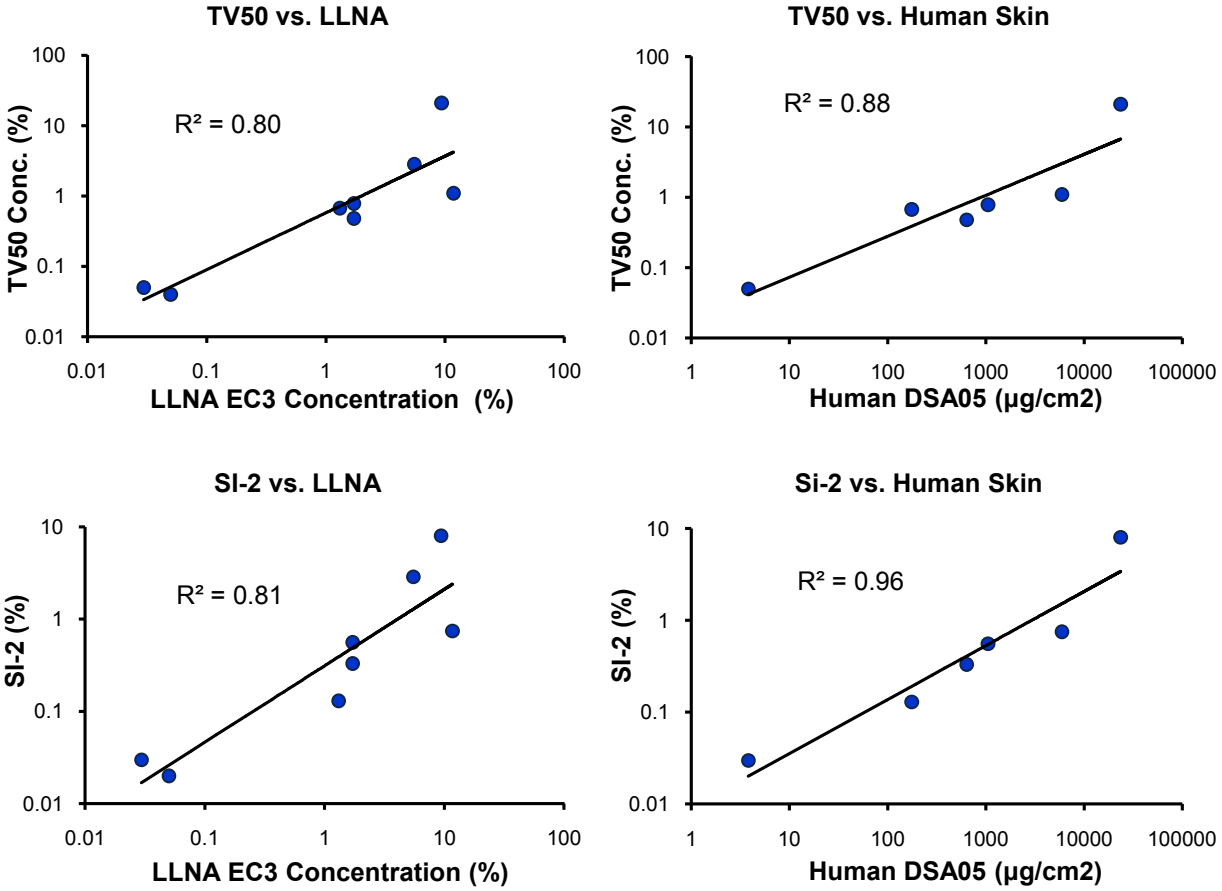


Figure 2a. Sensitizers in the IVSA



RESULTS (cont'd)

Figure 3. IVSA TV50 and IL-18 SI-2 data correlate with Murine LLNA EC3 and Human DSA_{05} data



CONCLUSIONS

- An SI of 2.0 was calculated to be the best fit cut-off to discriminate sensitizers from irritants and non-toxic chemicals.
- epiCS® IVSA correctly predicted sensitization potential with 89% Accuracy and 89% Sensitivity (18 chemicals).
- TV50 and IL-18 SI-2 data obtained from epiCS® tissues had very a high correlation (r^2=0.94).
- Using an SI-2 in this IVSA, data correlated better with Human DSA_{05} data (r^2=0.96) than with LLNA EC3 data (r^2=0.81).