

An Epidermal Equivalent Assay for identification and ranking potency of contact sensitizer

S. Gibbs¹, E. Corsini², E. Roggen³, O. Engelking⁴

¹VU university medical centre, Amsterdam (VUMC), The Netherlands; ²Università degli Studi di Milano (DiSFeB), Italy; ³RsMC; ⁴CellSystems, Germany

INTRODUCTION

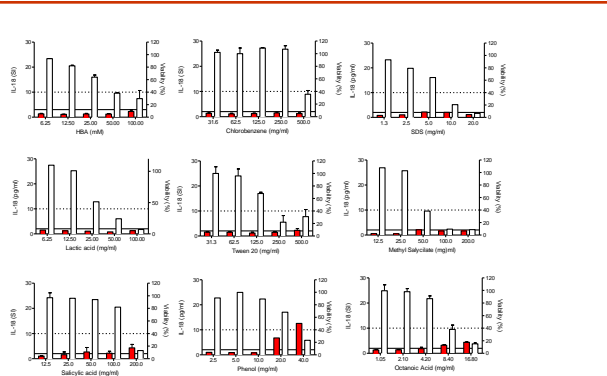
The aim of this study is to determine whether a single epidermal equivalent assay can identify sensitizers by their ability to release IL-18 into culture supernatant after topical exposure to sensitizers and also to rank sensitizers according to their potency which correlates to the irritant properties of the chemical.

Such an assay would be an added benefit for chemicals which are difficult to dissolve in culture medium.

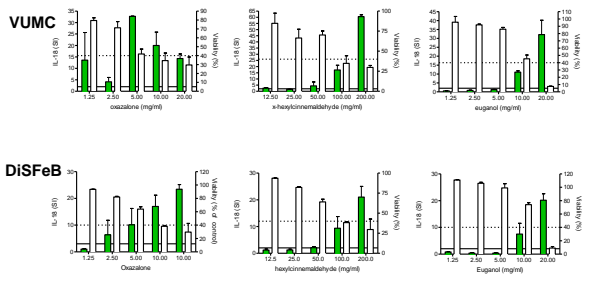
METHODS

- Topical exposure of the epidermis equivalent (EE) epiCS® (CellSystems, Germany) to sensitizers (11 coded) and non sensitizers (non coded) for 24 hours according to EE potency assay SOP
- determine amount of IL-18 released into supernatant after sensitizer or non-sensitizer exposure (yes/ no sensitizer)
- determine chemical concentration which results in 50 % decrease in viability (MTT assay) = EC50 value for sensitizers (sensitizer potency)

RESULTS



Topical exposure to non sensitizers results in NO dose dependent release of IL-18 into culture supernatants. 9 non coded non sensitizers tested. Red bar = IL-18 release (SI); white bar = viability (MTT assay).



Topical exposure to sensitizers results in a dose dependent release of IL-18 into culture supernatants. 11 coded sensitizers tested (2 not tested due to interference with MTT assay – cobalt chloride and PPD). Green bar = IL-18 release (SI); white bar = viability (MTT assay).

CONCLUSION

In this first study, sensitizers can be identified by IL-18 release into culture supernatants using a prediction model of 5 fold increase in IL-18 release at viability ranging from 5% - 40 % after topical chemical application. The same cultures can also be used to rank sensitizer potency using an EC50 value. The stronger the sensitizer the lower the EC50 value.

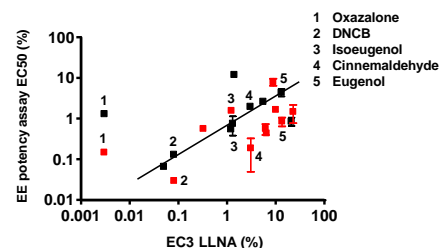
PREDICTION MODELS

Chemical	Prediction model 1 IL18 SI ≥ 5 at ≥ EC5		Prediction model 2 IL18 SI ≥ 5 at ≥ EC5 and ≤ EC40	
	VUMC	DiSFeB	VUMC	DiSFeB
extreme				
oxalalone	32.7±2.7	23.4±2.4	32.7±2.7	23.4±2.4
DNCB	26.2±4.2	16.8±1.8	26.2±4.2	16.8±1.8
strong				
formaldehyde	6.7±5.5	0.9±0.85 ^a	6.7±5.5	-
moderate				
phenylacetonealdehyde	35.6±20.1	2.3±1.1 ^b	35.6±20.1	-
isoeugenol	47.0±31.7	14.6±19.5	47.0±31.7	14.6±19.5
cinnamaldehyde	108±128	44.4±9.6	108±128	44.4±9.6
citral	21.0 ±9.5	16.5±0.1	21.0 ±9.5	16.5±0.1
2-mercaptobenzothiazol	59.7±0.9	3.5±1.9 ^b	59.7±0.9	-
weak				
x-hexylcinnamaldehyde	60.5±2.0	21.1±5.6	60.5±2.0	21.1±5.6
eugenol	32.1±11.4	20.2±3.4	32.1±11.4	20.2±3.4
benzocaine	19.8±27.2	18.4±2.0	19.8±27.2	18.4±2.0
Non sensitizers				
SDS	2.2±0.1		2.2±0.1	
octanoic acid		3.5±1.9		3.5±1.9
lactic acid	1.5±0.3		1.5±0.3	
phenol	12.6±5.6	1.1±0.1	12.6±5.6 ^b	1.1±0.1
methylsalicylate	2.1±0.5		2.1±0.5	
salicylic acid		4.3±1.9		4.3±1.9
Tween 20		2.3±1.0		2.3±1.0
chlorobenzene		1.5±0.3		1.5±0.3
HBA		2.3±1.2		2.3±1.2

^a viability ≥ 90 %; ^b viability is > 50 %, EC5 and EC40 = 5 % and 40 % cell viability respectively. False positives and negatives in the prediction models are shown red. Values obtained from chemicals which do not fulfill the acceptance criteria of the prediction model are not shown (-).

Prediction model 1: IL-18 SI 5 x at ≥ EC5, VUMC has only 1 false positive. DiSFeB has 3 false negatives. DiSFeB's false negatives can be explained by low toxicity being reached in the dose response which is needed to release intracellular IL18 into supernatant. Conclusion – at least 60 % cytotoxicity is required in this assay. Prediction model 2: IL-18 SI 5 x at ≥ EC5 and ≤ 40, VUMC has only 1 false positive and DiSFeB has 100 % accuracy.

Potency: correlation of EE-EC50 with LLNA. Results of this study with epiCS superimposed onto graph with previous results using VUMC-EE



■ Vumc EE dos Santos et al, TIV 2011
 ■ epiCS coded chemicals spearman r = 0.66; P value (two-tailed) = 0.03