Toxicology in Vitro 27 (2013) 1233–1246

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Artificial neural network analysis of data from multiple *in vitro* assays for prediction of skin sensitization potency of chemicals

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ARTICLE INFO

Article history:
Received 4 October 2012
Accepted 19 February 2013
Available online 1 March 2013

Keywords:
Skin sensitization
Cell-surface thiols
SH test
h-CLAT
Artificial neural network
Risk assessment

ABSTRACT

In order to develop *in vitro* risk assessment systems for skin sensitization, it is important to predict a threshold from the murine local lymph node assay (LLNA). We first confirmed that the combination of the human Cell Line Activation Test (h-CLAT) and the SH test improved the accuracy and sensitivity of prediction of LLNA data compared with each individual test. Next, we assessed the mutual correlations among maximum amount of change of cell-surface thiols (MAC value) in the SH test, CV75 value (concentration giving 75% cell viability) in a cytotoxicity assay, EC150 and EC200 values (thresholds concentrations of CD86 and CD54 expression, respectively) in h-CLAT and published LLNA thresholds of 64 chemicals. Based on the results, we selected MAC value and the minimum of CV75, EC150 (CD86) and EC200 (CD54) as descriptors for the input layer of an artificial neural network (ANN) system. The ANN-predicted values were well correlated with reported LLNA thresholds. We also found a correlation between the SH test and the peptide-binding assay used to evaluate hapten-protein complex formation. Thus, this model, which we designate as the "iSENS ver. 1", may be useful for risk assessment of skin sensitization potential of chemicals from *in vitro* test data.

1. Introduction

Predictive testing for potential to induce allergic contact dermatitis is a major component of the safety assessment of new ingredients to be employed in topically applied cosmetics and drugs. Animal model tests, such as the guinea pig maximization test (Magnusson and Kligman, 1969), have been employed as standard procedures for this purpose for many years. Recently, the murine local lymph node assay (LLNA) was adopted by the Organization for Economic Cooperation and Development (OECD) for skin sensitization hazard assessment (OECD Test Guideline 429). Furthermore, an exposure-based quantitative risk assessment (QRA) for skin sensitization was reported (Api et al., 2008). In QRA, the no expected sensitizing induction level (NESIL) is an important benchmark that is derived from human data (e.g., human repeated insult patch test (RIPT)) and animal data (e.g., LLNA) and is expressed as dose per unit area (e.g., μg/cm^2). EC3 values calculated from LLNA data are given as percent (%) or as dose per unit area (e.g., μg/cm^2). These units can be interconverted (Safford et al., 2011). If no human data are available, the thresholds of chemicals in LLNA, including EC3 values, can be used as a NESIL value based on a weight of evidence approach (Api et al., 2008). So, predicting LLNA thresholds is an important goal for skin sensitization risk assessment using *in vitro* methods.

Several *in vitro* skin sensitization assays that do not use animals have been reported, following the 7th amendment of the European Cosmetic Directive published in 2003. For example, the human cell line activation test (h-CLAT) (Ashikaga et al., 2006; Sakaguchi et al., 2006), U-937 assay (Python et al., 2007) and MUTZ-3 (Azam et al., 2006) are based on phenotypic changes of antigen-presenting cells (APCs), including dendritic cells (DCs), in response to hapten treatment (Aiba and Tagami, 1998; Aiba et al., 1997; Boisleve et al., 2004). The ARE assay (Natsch et al., 2009) and KeratinoSens (Emter et al., 2010) is based on stimulation of antioxidant response element (ARE)-dependent gene activity in a recombinant cell line. The SH test measures changes of cell-surface thiols induced by haptens as a model of activation of intracellular signal transduction (Suzuki et al., 2009). Peptide binding assay (Gerberick et al., 2007) reflects binding between protein and haptens, which is necessary for antigen presentation from APCs to T cells. Among them, peptide-binding assay, ARE assay and h-CLAT parameters have been reported to correlate with EC3 values in LLNA (Gerberick et al., 2004a,b; Natsch and Emter, 2008; Nukada et al., 2012). As regards h-CLAT, we and our co-researchers have reported that the EC150 value (estimated concentration giving a relative fluorescence

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http://dx.doi.org/10.1016/j.tiv.2013.02.013

Table 1
Chemical information including h-CLAT and SH test data. CAS no. = Chemical Abstract Service number. NS; non-sensitizing in LLNA. h-CLAT data are from the report by Nukada et al. (2012). In the case of RFI (%) in the SH test, mean values of RFI ± SD from at least three independent experiments are shown. RFI and MAC values were calculated as described in the text.

Test samples			LLNA			h-CLAT					CV75 (24 h) (μg/mL);(3)	Minimum values among (1), (2) and (3)	SH test					
Sample name		CAS no.	Potency category	EC3 values (%)	LLNA threshold (%)	Judgement (CD86 or CD54)	CD86		CD54				Judgement	RFI(%)	Conc. (μg/mL)	MAC values	Cell-surface thiols	References (LLNA)
							Judgement (CD86)	EC150 (μg/mL);(1)	Judgement (CD54)	EC200 (μg/mL);(2)								
Oxazolone	–	15646-46-5	Extreme	0.003	0.003	Positive	Positive	2.71	Negative	–	166.6	2.71	Positive	41.8 ± 6.4*	283.3	58.2	Decrease	Gerberick et al. (2004a,b)
DPCP	Diphenylcyclopropenone	886-38-4	Extreme	0.003	0.003	Positive	Negative	–	Positive	3.92	6.0	3.92	Positive	563 ± 126*	8.0	463	Increase	Gerberick et al. (2004a,b)
MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone (act. 1.5%)	26172-55-4	Extreme	0.005	0.005	Positive	Positive	2.21	Negative	–	3.2	2.21	Positive	37.7 ± 11.7*	385.0	62.3	Decrease	Gerberick et al. (2004a,b)
p-Benzoquinone	–	106-51-4	Extreme	0.0099	0.0099	Positive	Positive	2.68	Positive	2.25	4.3	2.25	Positive	58.9 ± 18.5*	5.0	41.1	Decrease	Gerberick et al. (2005)
TCSA	Tetrachlorosalicylanilide	1154-59-2	Extreme	0.04	0.04	Positive	Negative	–	Positive	1.2	2.6	1.2	Positive	43.2 ± 5.4*	87.0	56.8	Decrease	Gerberick et al. (2005)
DNCB	2,4-Dinitrochlorobenzene	97-00-7	Extreme	0.05	0.05	Positive	Positive	2.3	Positive	2.66	5.0	2.26	Positive	20.1 ± 4.8*	53.0	79.9	Decrease	Gerberick et al. (2004a,b)
Potassium dichromate	–	7778-50-9	Extreme	0.08	0.08	Positive	Positive	2.09	Positive	1.06	3.2	1.06	Positive	61.0 ± 3.2*	460	39.0	Decrease	Gerberick et al. (2005)
Glutaraldehyde (act. 50%)	–	111-30-8	Strong	0.1	0.1	Positive	Positive	2.78	Positive	2.7	5.3	2.7	Positive	70.4 ± 3.4*	17.8	29.6	Decrease	Gerberick et al. (2005)
1,4-Dihydroquinone	–	123-31-9	Strong	0.11	0.11	Positive	Positive	2.13	Negative	–	5.0	2.13	Positive	40.6 ± 19.3*	218	59.4	Decrease	Gerberick et al. (2005)
*p*PD	1,4-Phenylenediamine	106-50-3	Strong	0.16	0.16	Positive	Positive	2.09	Negative	–	36.7	2.09	Positive	32.3 ± 19.0*	5000	67.7	Decrease	Gerberick et al. (2005)
Phthalic anhydride	–	85-44-9	Strong	0.16	0.16	Negative	Negative	–	Negative	–	>400	400	Positive	72.1 ± 20.4*	2500	27.9	Decrease	Gerberick et al. (2004a,b)
Maleic anhydride	–	108-31-6	Strong	0.16	0.16	Positive	Negative	–	Positive	298.4	658.0	298.4	Positive	63.4 ± 12.9*	1800	36.6	Decrease	Basketter and Kimber (2011)
Benzoyl peroxide	–	94-36-0	Strong	0.30	0.3	Negative	Negative	–	Negative	–	41.0	41.0	Positive	69.5 ± 9.0*	33.3	30.5	Decrease	Basketter and Kimber (2011)
Propyl gallate	–	121-79-9	Strong	0.32	0.32	Positive	Negative	–	Positive	32.5	125.0	32.5	Positive	61.2 ± 10.2*	833	38.8	Decrease	Gerberick et al. (2007)
$CoCl_2$	Cobalt chloride	1332-82-7	Strong	0.4	0.4	Positive	Negative	–	Positive	35.5	208.3	35.5	Positive	40.8 ± 6.0*	5000	59.2	Decrease	OECD Test Guideline 429 (2010)
2-Aminophenol	–	95-55-6	Strong	0.4	0.4	Positive	Positive	0.89	Negative	–	6.0	0.89	Positive	70.0 ± 6.3*	2500	30.0	Decrease	Gerberick et al. (2005)