IATA for Skin Sensitisation Potential: 1 out of 2 or 2 out of 3

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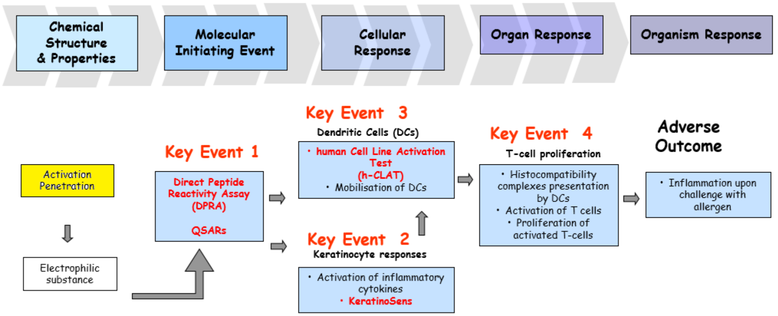
Abstract

To meet EU regulatory requirements and to avoid or minimise animal testing, there is a need for non-animal methods to assess skin sensitisation potential. Given the complexity of the skin sensitisation endpoint, there is an expectation that integrated testing and assessment approaches (IATA) will need to be developed which rely on assays representing key events (KEs) in the pathway. Three non-animal assays have been formally validated: the direct peptide reactivity assay (DPRA), the KeratinoSens™ assay and the h-CLAT assay. At the same time, there have been many efforts to develop IATA with the "2 out of 3" approach attracting much attention whereby a chemical is classified on the basis of the majority outcome. A set of 271 chemicals with mouse, human and non-animal sensitisation test data was evaluated to compare the predictive performances of the 3 individual non-animal assays, their binary combinations and the '2 out of 3' approach. The analysis revealed that the most predictive approach was to use both the DPRA and h-CLAT: 1. Perform DPRA – if positive, classify as a sensitiser; 2. If negative, perform h-CLAT – a positive outcome denotes a sensitiser, a negative, a non-sensitiser. With this approach, 85% (LLNA) and 93% (human) of the non-sensitiser predictions were correct, in contrast to the '2 out of 3' approach which had 69% (LLNA) and 79% (human) of non-sensitiser predictions correct.

Development of IATA



Aim

To evaluate the predictive performance of the 3 individual assays, their binary combinations and the '2 out of 3" approach (Urbisch et al. 2015)

Binary combination and '2 out of 3'

Binary combination – Test using assay A. If result is positive, the chemical is considered a sensitiser. If result is negative, test using assay B. If assay B is positive, then the chemical is considered a sensitiser. A positive in either assay is sufficient to classify a chemical as a sensitiser but negative outcomes in both assays are needed to classify as a non-sensitiser.

'2 out of 3' – Test using assay A and assay B. If both results are positive, the chemical is a sensitiser. If both results are negative, the chemical is a non-sensitiser. If the results disagree, test in assay C – if the result is positive, the chemical is a sensitiser, if negative, the chemical is a non-sensitiser.

Methods

The dataset for this study was compiled by EURL ECVAM (Asturiol et al., 2016).
It consisted of 271 chemicals with at least a result in one of the 3 non-animal test methods: DPRA, KeratinoSens™ and h-CLAT data.
The study dataset was complemented with LLNA outcomes and human response data. The LLNA outcomes were taken from submissions to EURL ECVAM as well as scientific publications whereas the human response data was taken from Basketter et al (2014).

Results: Performance characteristics (LLNA)

	DPRA	Kerat	h-CLAT	DPRA+ Kerat	DPRA+ h-CLAT	Kerat+ h-CLAT	2 out of 3
Sensitivity	82	76	80	94	97	97	86
Specificity	75	69	68	42	52	34	76
Accuracy	80	73	76	77	86	77	83
Probability that classed S is true S	90	76	86	77	86	76	89
Probability that classed NS is true NS	61	69	58	76	85	83	69
Number of chemicals	162	215	160	184	171	185	152

Results: Performance characteristics (Human)

	DPRA	Kerat	h-CLAT	DPRA+ Kerat	DPRA+h-CLAT	Kerat+h-CLAT	2 out of 3
Sensitivity	86	79	89	94	98	98	90
Specificity	90	73	70	61	65	48	83
Accuracy	87	77	83	84	89	82	88
Probability that classed S is true S	96	86	87	84	89	80	92
Probability that classed NS is true NS	73	63	73	82	93	92	79
Number of chemicals	71	79	76	75	76	79	74

Results: Best strategies

Of the 3 assays considered individually, the DPRA is marginally better than the other 2 for predicting the sensitising outcome in the LLNA. Combinations of the assays perform better than the individual assays.
The combination of DPRA + h-CLAT performs best in terms of correctly classifying sensitisers and non-sensitisers.

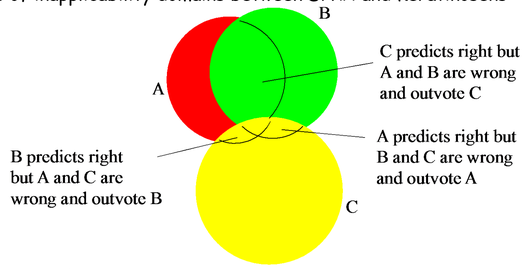
- For maximum confidence that a predicted sensitiser is really a sensitiser: Run the DPRA only (PPV – positive predicted value)
- For maximum confidence that a predicted non-sensitiser is really non-sensitising: Follow up a negative DRPA with the h-CLAT (NPV – negative predicted value)

Discussion

The performance characteristics raise the question of whether the assays themselves are really measuring the different key events within the AOP or whether the different protocols are merely compensating for each other's technical limitations. If all of the KEs need to occur, then only chemicals that are positive in all three assays should be classified as sensitisers. E.g. a chemical positive in DRPA, negative in KeratinoSens™ but positive in h-CLAT should be classified a non-sensitizer.

All three assays are best considered as reactivity assays, none of them being applicable for all chemicals but differing from each other to a greater or lesser extent in terms of their domains of applicability.

Greater overlap of inapplicability domains between DPRA and Keratinosens™



DPRA and Keratinosens™ are like A and B; h-CLAT is like C

Conclusions

- A DPRA+h-CLAT combination of assays performs best in terms of correctly classifying for sensitisers and non-sensitisers on the basis of this dataset
- The performance characteristics suggest that the assays are really assessing the ability of a chemical to react with protein.
- None of the assays completely covers the diversity and range of reaction chemistry leading to protein binding
- Since their inapplicability domains differ, combinations of assays are able to outperform individual assays

References

Asturiol et al 2016 TIV in press; Basketter et al. 2014 Dermatitis 25: 11; Urbisch et al. 2015: RTP 71: 337