A Comparative Evaluation of In Vitro Skin Sensitisation Tests: The Human Cell-line Activation Test (h-CLAT) versus the Local Lymph Node Assay (LLNA)

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Summary — We previously developed the human cell-line activation test (h-CLAT) in vitro skin sensitisation test, based on our reported finding that a 24-hour exposure of THP-1 cells (a human monocytic leukaemia cell line) to sensitisers is sufficient to induce the augmented expression of CD86 and CD54. The aim of this study is to confirm the predictive value of h-CLAT for skin sensitisation activity by employing a larger number of test chemicals. One hundred chemicals were selected, according to their categorisation in the local lymph node assay (LLNA), as being: extreme, strong, moderate and weak sensitisers, and non-sensitisers. The correlation of the h-CLAT results with the LLNA results was 84%. There were some false negatives (e.g. benzoyl peroxide, hexyl cinnamic aldehyde) and some false positives (e.g. 1-bromobutane, diethylphthalate). Eight out of the 9 false negatives (89%) were water-insoluble chemicals. The h-CLAT could predict not only extreme and strong sensitisers, but also moderate and weak sensitisers, though the detection rates of weak sensitisers and non-sensitisers were comparatively low. Some sensitisers enhanced both CD86 and CD54 levels, and some enhanced the level of only one of them. The use of the combination of CD86 and CD54 induction as a positive indicator, improved the accuracy of the test. In conclusion, the h-CLAT is expected to be a useful cell-based in vitro method for predicting skin sensitisation potential.

Key words: human cell-line activation test, skin sensitisation, THP-1.

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Introduction

Because of increasing social concerns about animal welfare, many alternative test methods have been proposed, especially for skin sensitisation testing (1). One of the most important approaches for developing alternative methods for skin sensitisation testing has been to measure phenotypic changes, such as the expression of CD86 or CD54 on the surface of dendritic cells (DCs) exposed to test agents (2, 3). However, the use of DCs is problematic, because the effects of chemicals on the surface phenotype of DCs was found to be dependent on the source of peripheral blood — that is, the effect varied from donor to donor (2, 4). Furthermore, peripheral blood as a source of DCs is not necessarily readily available. Therefore, we tested the human leukaemia cell line THP-1 as a substitute for DCs, and concluded that THP-1 cells, which show enhanced CD86 and/or CD54 expression when treated with sensitisers, can be used for in vitro skin sensitisation testing (5, 6). We named this test the human cell-line activation test (h-CLAT). In previous studies, we optimised the test conditions (7, 8), and we established that the h-CLAT can predict the sensitisation potential of a number of preservatives which are well-known sensitisers (9). Another group has also reported that THP-1 is a promising in vitro model for assays aimed at predicting the sensitisation potentials of chemicals, in that this cell-line is easy to handle and offers a number of practical advantages (10). Thus, the h-CLAT is expected to be a useful tool as a component of an in vitro test battery for predicting skin sensitisation potential.