but, as in chronic cutaneous wounds, the epithelium remained negative.

In conclusion, successful wound healing is accompanied by tightly scheduled expression of metalloproteinases and their inhibitors. Their imbalance may delay wound healing and result in chronic ulcers. MMPs and TIMPs are also involved in both tissue destruction and mucosal reparative processes during the course of inflammatory bowel diseases.

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Predictive Testing for Contact Allergy. Comparison of Some Guinea Pig and Mouse Protocols Including Dose-Response Designs

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Contact allergy (delayed hypersensitivity) may develop as a result of skin exposure to contact allergens (haptens) and can lead to allergic contact dermatitis. The purpose of this study was to evaluate some predictive animal test methods for contact allergens. It was done with the aim that the test methods giving the clinically most relevant results should be used in risk assessment of chemicals and in research.

Helen Wahlkvist defended her thesis on January 14, 2000 at the Unit of Dermatology and Venereology, Department of Medicine, Karolinska Institute. Faculty Opponent was Professor Klaus E. Andersen, Odense University Hospital, Odense, Denmark and Chairman was Associate Professor Carola Lidén, Occupational and Environmental Dermatology , Stockholm County Council, Stockholm, Sweden.

A slightly modified multi-dose-response induction protocol was evaluated with two model contact allergens when applied to three guinea pig predictive test methods. The protocol was easily applied to the cumulative contact enhancement test (CCET) and the Freund's complete adjuvant test (FCAT), which have only one induction route. However, for the

guinea pig maximization test (GPMT) with two induction routes, the topical doses at induction interacted with the logistic model. The protocol would benefit from further development and some modifications are suggested. Calculations of the estimated concentration sensitizing 50% of the animals (EC_{50}) improves the possibility for properranking of contact allergens and augments the information used in risk assessment. The calculated EC_{50}-values for the model allergens were: 0.00045% in the FCAT, 0.0025% in the GPMT and 0.0042% in the CCET for potassium dichromate ($K_2Cr_2O_7$), and 0.068% in the guinea pig maximization test, 0.89% in the FCAT and 1.8% in the CCET for hydroxycitronellal (HC).

A multi-dose-response induction protocol was applied on a modified mouse ear swelling test (MEST) and evaluated with four contact allergens and one irritant. This protocol could detect the moderate to strong contact allergens as sensitizers, but not one (HC) of the two weak contact allergens. The irritant (negative control) gave a 'negative' response. The EC_{50}-values calculated for the three detected allergens were 0.002% for oxazolone, 0.03% for $K_2Cr_2O_7$ and 0.7% for methyldibromo glutaronitrile (MDBGN).

The murine local lymph node assay (LLNA) is a predictive test method, but its ability to discriminate between allergens and irritants has been questioned. Eight contact allergens and six irritants were investigated in the evaluation of the LLNA. The moderate to strong allergens gave clearly 'positive' results (stimulation index (SI) = 12.8 – 39.9), but one weak allergen (benzocaine) was not classified as a sensitizer (SI < 3). The irritants tested, i.e. chloroform/methanol, methylsalicylate, nonanoic acid, oxalic acid, sodium dodecyl sulfate (SDS), Triton X-100, however, gave also 'positive' results (SI = 5.0–10.7), not distinguishable from the results with weak and moderate contact allergens (SI = 3.4–17.1). The addition of 10% SDS could not be used to reduce the induced proliferation due to irritation from the test chemicals, nor could an alternative choice of vehicle.

The allergenicity of a preservative, i.e. Euxyl K 400, and one of its ingredients, MDBGN, was investigated in three different animal predictive test methods, and patch testing in dermatitis patients was performed for comparison. The CCET using a multi-dose-response induction protocol (EC_{50} = 1.9 % for MDBGN) and the LLNA (SI = 7.4–7.9 for MDBGN and 8.4–12.0 for Euxyl K 400) confirmed the sensitization potential of the substance based on dermatitis patients patch test results (total frequency varied between 0.9–1.8%). However, the results from the GPMT were not statistically significant.

In conclusion, even though the LLNA and the MEST have some advantages compared to the guinea pig test methods concerning speed, labour-intensiveness and cost, and the use of an objective end point, the methods are at present not capable of replacing the predictive guinea pig test methods. Both the LLNA and MEST gave a lower sensitization rate with the weak and moderate contact allergens tested than the guinea pig test methods did. The MEST is judged to be less capable of detecting potential contact allergens than the LLNA, but on the other hand no false 'positive' reactivity with the irritant tested was seen. Dose-response designs of predictive test methods increase the amount of information obtained from each sensitization study and should be considered for inclusion in the protocols used when the sensitizing potential of a substance is investigated.

Investigators are advised to select the predictive test method with the induction procedure that is most relevant for the prospected use of the substance being tested. A test method with a particular induction route may be more suitable for testing a substance than one of the recommended methods, so there is also a possibility to use other available standardized predictive animal test methods. However, that predictive test methods have a varying capacity to detect the sensitizing potential of a substance is evident.

Key words: contact allergens, contact allergy, cumulative contact enhancement test, dose-response, evaluation, Freund's complete adjuvant test, GPMT, local lymph node assay, mouse ear swelling test, patch test, predictive testing, statistical analysis.