Contact Dermatitis 1993: **28:** 81–83

Allergic contact reaction to dexpanthenol: lymphocyte transformation test and evidence for microsomal-dependent metabolism of the allergen

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In a patient with contact dermatitis, dexpanthenol was found to be the causative allergen. There was a positive reaction to dexpanthenol on patch testing. Controls did not show any positive reactions to dexpanthenol on patch testing. Additionally, an LTT was performed. After preincubation with dexpanthenol-modified microsomes, we observed an increase in lymphocyte proliferation to dexpanthenol, in comparison to dexpanthenol without microsomes, suggesting that microsomal metabolism plays a rôle in the pathogenesis of dexpanthenol sensitization, because microsomes are known to posses drug metabolizing enzymes such as cytochrome P450.

Key words: dexpanthenol; patch testing; lymphocyte transformation test; cytochrome P450; allergic contact dermatitis; medicaments.

Accepted for publication 16 June 1992

Dexpanthenol is a widely used constituent of several ointments, eyedrops, injections and tablets. Local treatments of rhinitis, conjunctivitis and sunburn are recommended indications and wound healing (ulcers, burns, bed sores and excoriations) is improved by dexpanthenol (4). Although it is a frequently applied substance, there are only very rare reports of sensitization to dexpanthenol (1–6, 9, 10).

A recently observed patient with contact dermatitis after using Bepanthen® Creme, and a positive patch test reaction to dexpanthenol, prompted us to perform a lymphocyte transformation test with dexpanthenol-modified microsomes, to investigate whether the reaction was a specific immunologically T-cell-dependent reaction and whether there was evidence that microsomal-dependent metabolism meight play a rôle in sensitization.

Materials and Methods

Patch testing

Patch testing was carried out with Bepanthen® Creme and dexpanthenol 1% in glycerol, according to ICDRG recommendations (11). 23 volunteers without a history of allergic reactions to medicaments served as controls.

Lymphocyte transformation test

Cell preparation (7). Human peripheral mononuclear cells were separated by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) from heparinized venous blood. The lymphocyte layer was transferred and washed 3×, 2× with Hanks' balanced salt solution (Difco, Detroit Michigan, USA) and 1× with RPMI 1640 (Gibco Biocult, Glasgow, Scotland).

Preparation of microsomes. NMRI-mice were shaved and for 3 days smeared with liquor carbonis detergens. The hepatic microsomal suspension was isolated by differential ultra-centrifugation (8).

Microcultures (200 µl)

MC+	S in RPMI 1640
MC+D+	S in RPMI 1640
MC+D+M+N+	S in RPMI 1640
MC+M+N+	S in RPMI 1640
MC+PHA+	S in RPMI 1640

Abbreviations

MC:	mononuclear cells 10^6/ml
S:	autologous serum 10%
D:	dexpanthenol 200, 20, 2, 0.2 µg/ml
M:	murine microsomes 0.01 mg/ml
N:	NADPH 0.016 mg/ml
PHA:	phytohaemagglutinin

After 6 h of incubation, the cultures were centrifuged, the supernatant removed and replaced by RPMI 1640 medium with autologous serum.

Proliferation assay. Proliferation was measured by ^{3}H-thymidine incorporation after 3, 5 and 7 days of total incubation time. The result was expressed as a stimulation index (SI):

$$SI = \frac{\text{cpm lymphocytes with dexpanthenol}}{\text{cpm lymphocytes without dexpanthenol}}$$

Results

Patch testing

Patch testing yielded a ++ reaction to dexpanthenol and a +++ reaction to Bepanthen® Creme (Table 1). The 23 controls did not show any positive reactions to Bepanthen® Creme or dexpanthenol.

Lymphocyte transformation test

For the LTT, various concentrations of dexpanthenol, different durations of cultivation and media were chosen.

2 μg dexpanthenol/ml induced peak values of the patient's lymphocytes with an SI of 2.2 (Table 2). The controls ($n = 4$) showed no lymphocyte proliferation at all (Table 2).

After incubation for 5 days and addition of murine liver microsomes, the peak value increased to an SI of 3.0 (Table 2). Control cultures with microsomes but without dexpanthenol did not induce lymphocyte proliferation (Table 2). The same was the case in 4 healthy volunteers who had no reaction to microsomes with or without dexpanthenol (Table 2).

Discussion

History, patch testing and LTT suggested the diagnosis of an allergic reaction to dexpanthenol in this patient.

Patch testing showed a typical crescendo reaction to both Bepanthen® Creme and its constituent dexpanthenol. None of the 23 controls showed any positive reactions, ruling out irritant reactions.

In the lymphocyte transformation test, peak values appeared after 5 days of cultivation at a concentration of 2 μg dexpanthenol/ml. Higher concentrations showed toxic effects (SI < 1) and a lower concentration was insufficient to cause the same extent of lymphocyte proliferation.

To assess the influence of drug metabolism on sensitization to dexpanthenol, murine liver microsomes were added. Increased lymphocyte proliferation in the presence of microsomes suggests that microsomal-dependent metabolism participates in the formation of the ultimate antigen. To exclude non-specific stimulation by microsomes themselves, control cultures with microsomes but without dexpanthenol were performed. These did not induce any lymphocyte proliferation. Further controls with 4 healthy volunteers also did not show any proliferation to dexpanthenol or to dexpanthenol with microsomes.

Thus the reaction observed is likely to be a specific T-cell-dependent reaction enhanced by microsomal-dependent metabolism of the antigen. In microsomes, including cutaneous microsomes (8), cytochrome P450 isoenzymes are present and might play a rôle in the chemical activation of drugs or xenobiotica. Such chemical activation enables many compunds to bind to macromolecules, which is considered a prerequisite for haptens to become allergens. The reaction observed in the lymphocyte transformation test suggests that such a mechanism plays a rôle in the rare allergic reactions to dexpanthenol.

Table 1. Patch test results

Allergen	D1	D2	D3	D4
patient with contact dermatitis				
Bepanthen® Creme	0	+	+++	++
dexpanthenol 1% in glycerol	0	(+)	++	++
23 control patients				
Bepanthen® Creme	0	0	0	0
dexpanthenol 1% in glycerol	0	0	0	0

Table 2. Lymphocyte tranformation test results

5-day cultures	4 controls		patient
	(μg/ml)	mean ± standard error	mean
MC + D	0.2	1.1 ± 0.2	1.5
	2	1.2 ± 0.2	2.2
	20	1.0 ± 0.1	0.9
	200	1.0 ± 0.1	0.6
MC + M + N + D	0.2	1.7 ± 0.2	2.3
	2	1.4 ± 0.4	3.0
	20	1.3 ± 0.1	1.8
	200	1.4 ± 0.1	1.8
MC + M + N		1.1 ± 0.1	1.2
MC + PHA		44.6 ± 5.7	58

Abbreviations:
MC: Mononuclear cells 10^6/ml.
D: Dexpanthenol 200, 20, 2, 0.2 μg/ml.
M: Murine microsomes 0.01 mg/ml.
N: NADPH 0.016 mg/ml.
PHA: phytohaemagglutinin.

Acknowledgement

This work was supported in part by a grant from the Deutsche Forschungsgemeinschaft (DFG – Me 939/1-1-).

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