

## THE INFLUENCE OF TOPICAL STEROID APPLICATION ON TUBERCULIN SKIN REACTIONS IN HEALTHY PERSONS

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**Abstract.** Thirty-seven healthy persons were studied in order to evaluate the influence of topical steroid application on tuberculin skin reactions. Four areas measuring 4 cm in diameter were each treated with 50 µg of hydrocortisone cream 1%, or 50% µg of halcinonide (Halog<sup>®</sup>) cream 0.1%, or 50 µg of unguentum cetacei simplex (cold cream), or not treated. The creams were applied once daily for 3 days before and one day after a tuberculin skin test. After 24 and 48 hours the areas of induration were measured. We observed that application of unguentum cetacei simplex increased the size of the induration at the 24-hour reading, but not after 48 hours. Hydrocortisone cream 1% gave the same effect, whereas halcinonide cream (Halog<sup>®</sup>) 0.1% caused ischaemia of the skin and reduced the induration of the skin test after 24 hours, but not after 48 hours. In 12 persons we found that simple rubbing of the skin with halcinonide cream base did not affect the size of the tuberculin skin reaction. In the present study we found that even very potent local steroid application on intact skin could only delay the development of tuberculin skin reactions, but could not diminish their size.

**Key words:** Tuberculin skin reactivity; Topical steroid

The introduction of topical steroid preparations has created major improvements in dermatology. The clinical benefit of local steroid application to patients with eczematous diseases cannot be doubted. However, several aspects of the activity of steroid preparations are still unsolved, such as the exact mechanism by which steroids influence the inflammatory or allergic reactions in the skin, or with which methods we should investigate the biological efficacy of steroid preparations (for reviews, see 7, 13).

When considering whether a decreased ability of atopic patients to express tuberculin skin reactions could be due to previous topical steroid treatment, we decided to investigate the influence of local steroid application on the development and expression of tuberculin skin test reactions in healthy persons.

## METHODS

Twenty-nine members of the staff at our department participated in the investigation. None were ill at the time of the study and none had previously used topical steroids. Four persons were later excluded because they were tuberculin negative. The mean age of the group was 36.7 years (range 19 to 62 years). There were 17 women and 8 men.

Four circular areas of 13 cm<sup>2</sup> (diameter 4 cm) were marked on the volar sides of the forearms, two on each arm. The distance between the centres of two adjacent areas was 7 cm. Marks with fluorescent stain were applied so that the same locations could be traced accurately every day. 50 µg of various creams was then applied once daily and rubbed into the skin with 50 circular movements of a fingertip.

We used hydrocortisone cream 1%, halcinonide (Halog<sup>®</sup>) cream 0.1%, unguentum cetacei simplex (cold cream), or no application at all. Cold cream was used as an indifferent cream, because hydrocortisone cream base and halcinonide cream base were not available to us at the beginning of the investigation.

Each person received a randomly selected application schedule, which was used throughout the study. In these schedules the pattern of application was shifted so that left or right arm, upper or lower locations, were equally distributed for each particular cream. After three consecutive daily applications all persons received four tuberculin skin tests with purified protein derivative of tuberculin, 2 I. U. each (Statens Seruminstitut, Copenhagen), one test in the centre of each area. At 24 and 48 hours after skin testing, the areas of induration were read, in most cases by a laboratory technician without any knowledge of the purpose of the investigation. After marking the area of induration with ink, the stain, and thus the area, was transferred to ordinary paper using tape. The areas were measured using a Hewlett-Packard Calculator Digitizer, model 9107A, and expressed in cm<sup>2</sup>. Following the 24-hour reading a fourth and final application of cream was made.

In order to study the influence of simple rubbing of the skin and of halcinonide (Halog<sup>®</sup>) cream base on the tuberculin skin reactivity, we looked at another group of staff and in-patients with crural ulcers, 7 women and 5 men (mean age 44 years, range 20 to 63 years). The investigations in this group followed the same lines as described above except that the four areas were (i) untreated, (ii) rubbed with a fingertip (50 circular movements) as if cream were being applied, (iii) treated with 50 µg hal-

Table I. Mean areas (cm<sup>2</sup>) of tuberculin skin reactions (2 I. U.) in healthy persons following topical application of cold cream or halcinonide cream (Halog<sup>®</sup>) 0.1%

Number of healthy persons: 25. Mean values  $\pm$  1 S.D.

Readings	No application	Unguentum cetacei simplex	Cremor Halog
24 hours	1.96 $\pm$ 3.33	2.53 $\pm$ 3.84 <sup>a</sup>	1.52 $\pm$ 2.25 <sup>b, c</sup>
48 hours	3.59 $\pm$ 4.24	3.85 $\pm$ 4.65	3.24 $\pm$ 4.85

'Ung. cet. simplex' reaction was significantly larger than 'no application';  $p < 0.05$ .

'Cremor Halog' reaction was significantly smaller than 'ung. cet. simplex';  $p < 0.01$ .

'Cremor Halog' reaction was not significantly different from 'no application';  $0.05 < p < 0.10$ .

cinonide (Halog<sup>®</sup>) cream base, or (iv) treated with 50  $\mu$ g halcinonide (Halog<sup>®</sup>) cream 0.1%. The tuberculin skin testing was done using 1 I. U. of tuberculin.

The statistical calculations were done using Wilcoxon's non-parametric test for pair differences, as the results were not normally distributed.

## RESULTS

The coefficient of variations was 5.1% for twenty consecutive measurements on the same tuberculin skin reaction. Twenty measurements with the planimeter on the area from one tuberculin skin reaction gave a coefficient of variation on 2.9%.

We compared the areas of skin reaction which had received identical treatment, but were located differently. There was no significant difference between the results, depending upon the anatomical position of the test site. This accords with previous observations (14). We could therefore compare our results regardless of the anatomical position of the skin tests.

When cold cream was rubbed into the skin a significantly larger reaction occurred after 24 hours (29% increase) compared with the reaction in untreated skin (Table I). In a smaller group of persons

Table II. Mean areas (cm<sup>2</sup>) of tuberculin skin reactions (2 I. U.) in healthy persons following application of hydrocortisone cream 1%

Number of healthy persons: 18. Mean values  $\pm$  1 S.D.

Readings	No application	Cremor hydrocortisone
24 hours	1.31 $\pm$ 1.34	1.67 $\pm$ 1.84 <sup>a</sup>
48 hours	3.59 $\pm$ 4.91	3.63 $\pm$ 5.90

'Cremor Hydrocortisone' reaction significantly larger than 'no application';  $p < 0.02$ .

we found that hydrocortisone cream 1% did not diminish the size of a tuberculin skin reaction; actually the reaction was significantly larger than on untreated skin after 24 hours, but not after 48 hours (Table II).

When halcinonide (Halog<sup>®</sup>) treated areas were compared with untreated areas no statistically significant reduction was found ( $0.05 < p < 0.10$ ; 24-hours reading).

To eliminate any possible mechanical influence on the development of a tuberculin skin reaction, we compared steroid treated areas with the cold

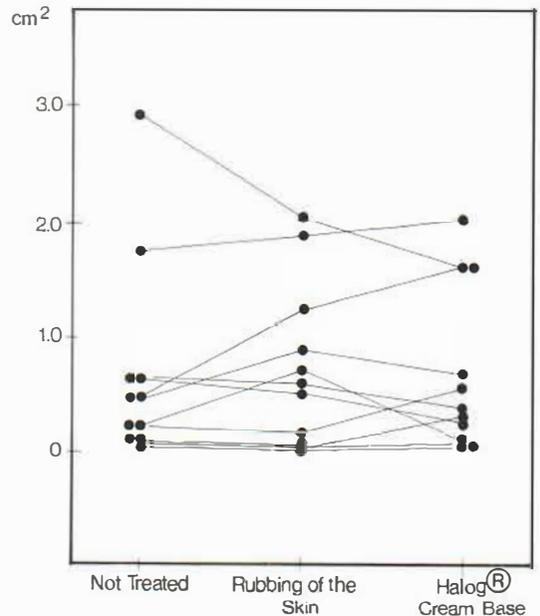


Fig. 1. The areas of tuberculin skin reactions (1 I. U.) in 12 healthy persons, when the skin was untreated, rubbed with 50 circular movements of a fingertip, or rubbed with halcinonide (Halog<sup>®</sup>) cream base. The readings were made after 24 hours. The ordinate shows the area in cm<sup>2</sup>.

Table III. *Tuberculin skin reactions (I. U) in 12 healthy subjects following various treatments of the skin*

The results are expressed in cm<sup>2</sup>. Skin rubbing means 50 circular movements with a fingertip. Halog<sup>®</sup> cream base and Halog<sup>®</sup> were applied as 50 µg and rubbed in to the skin with 50 circular movements of a fingertip

	No treatment	Skin rubbing	Halog <sup>®</sup> cream base	Halog <sup>®</sup>
24 hours				
Mean value ( $\bar{x}$ )	0.71	0.71	0.71	0.52
Range	0.10-2.90	0.03-2.06	0.06-2.06	0.03-1.87
48 hours				
Mean value ( $\bar{x}$ )	3.23	2.84	3.29	3.16
Range	0.65-7.61	0.58-6.06	0.52-7.10	0.13-8.26

cream treated area. We observed that halcinonide (Halog<sup>®</sup>) 0.1% cream was able to reduce significantly the size of the skin reactions at the 24-hour readings, but not after 48 hours (Table I).

In 2 persons we followed the course of the tuberculin skin reactions until disappearance. In both we found that halcinonide (Halog<sup>®</sup>) cream 0.1% only reduced the size of the reaction at the 24-hour reading, but not later. Also, the Halog-treated reactions did not disappear before the reactions in untreated skin.

In the second part of the study we looked for a possible mechanical influence on the development of tuberculin skin reactivity and whether halcinonide (Halog<sup>®</sup>) cream base could induce any changes. The results are presented in Table III. We found no influence on the tuberculin skin reactivity. Halcinonide (Halog<sup>®</sup>) cream treated areas had reduced tuberculin skin reactivity after 24 hours, though the difference was not found statistically significant (Table III).

## DISCUSSION

When hydrocortisone or Compound F was introduced into dermatology, several authors studied its effect on the development of delayed hypersensitivity (type IV) reactions in the skin (1, 2, 6, 11, 12). In all investigations it was found that hydrocortisone could not affect the size of a tuberculin skin reaction or patch test, even when the test area was occluded (1). If, however, the normal epidermis was removed (1), or the drug was injected intradermally at the patch test site (12), then an inhibition occurred. This finding of an inability of locally applied hydrocortisone to inhibit a type IV reaction obviously differed from its clinical effect on eczematous skin diseases, where lesions improved

significantly even after a short period of treatment (2). This fact apparently led to a loss of interest in tuberculin skin reactions and locally applied steroids.

In the present study we found that rubbing of the skin with unguentum cetacei simplex (cold cream) increased the size of the tuberculin skin reaction after 24 hours, but not after 48 hours. The slight mechanical friction apparently could not alter the tuberculin skin reaction. An earlier occurrence of an immunological type IV reaction might be due to a temporary vasodilatation from some of the components in the ointment. This effect might also explain why a possible inhibitory effect of hydrocortisone cream 1% was overwhelmed, leading to the paradox that the size of the tuberculin skin reaction was increased at the 24-hour reading. It is also quite remarkable that halcinonide (Halog<sup>®</sup>) cream 0.1%, which is considered to be one of the most potent topical steroid preparations available, cannot reduce the size of a tuberculin skin reaction in normal skin, but can inhibit the speed of its development.

What happens when a tuberculin skin reaction takes place? Two important factors are vasodilatation and lymphocyte accumulation. When antigen triggers antigen-reactive lymphocytes, these cells release factors which, through the mast cells, induce vasodilatation. If the mast cells are blocked, then a significant inhibition of the reaction occurs (5). This indicates that vasodilatation is of importance for the development of a type IV reaction. Apart for their influence on mast cells, lymphocytes also release lymphokines such as migration inhibition factor and several other chemotactic factors. If antibodies to migration inhibition factor are injected locally around the site for a later tuberculin skin test, then the reaction will be abolished completely (4).

It is not possible for us to say by which mechanism halcinonide (Halog<sup>®</sup>) cream 0.1% delays the tuberculin skin reaction. This topical steroid induces a strong, visible vasoconstriction and the initial reduction in the induration of the tuberculin skin test may be due to this action. From the two long-term observations, it seems that the accumulation and activity of the lymphocytes run a normal course.

Our present method of topical steroid application gave insufficient concentration of steroid in the dermis for a profound inhibition of tuberculin skin reactions. The method is exactly comparable to the method of Feldman & Maibach (3), who found that the penetration of steroid through normal epidermis is approximately equivalent to 1% of the applied amount of steroid. They also observed a large variation in absorption between different persons. Healthy epidermis is thus an efficient barrier for steroid penetration. If epidermis is removed, then even hydrocortisone cream 1% can inhibit a tuberculin skin reaction (1).

At present the only clinical method of assaying the biological activity of topical steroids in man is the vasoconstriction assay (8, 9, 10). Further studies, including stripping of the epidermis, will reveal whether the tuberculin skin test can be used as *in vitro* test for topical steroids and elucidate their biological influence on a type IV reaction involving both vasodilatation and a specific immunological reaction.

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