

HYDROCORTISONE (CORTISOL) CONCENTRATION AND PENETRATION GRADIENT

G. Kranz, H. Schaefer and A. Zesch

From the Department of Dermatology, The Free University of Berlin, Rudolf Virchow Hospital, Berlin

Abstract. Hydrocortisone (Cortisol) was incorporated into four different ointments at four concentrations (0.1% 0.3%, 1%, and 3%) and the amounts of the drug penetrating from these preparations into the different layers of excised skin were investigated. The effect of removing the horny layer on the penetration was also determined. Below a level of 1% ointments with intact horny layer and 0.3% with removed layer, a change in the ointment concentration alters the tissue concentration in the ratio 1:1. Above this level doubling of the concentration in the ointments causes an increase of tissue concentrations of only 20-50%. The removal of the horny layer increases the dermal concentrations 100 fold at each ointment concentration. Furthermore, a vasoconstriction test was performed with the different ointments. An increase in the concentrations above the 1% level failed to increase the effect on the vascular system of the skin. The in vitro and in vivo results are discussed with respect to the consequences on affected skin and the therapeutic efficacy of steroid preparations of different concentrations.

Key words: Hydrocortisone (Cortisol); Skin penetration

Clinical studies on hydrocortisone (Cortisol)-containing ointments have shown that the optimal therapeutic concentration of the drug is about 1%. The clinical effectiveness of a drug is the result of the interplay of several independent factors such as drug penetration, rate of removal (excretion or metabolic modification), in-situ concentration of the active agent etc. Therefore it is clear that when the clinical efficacy of a drug is investigated, using several preparations differing in their drug concentrations, no conclusions can be drawn as to the actual drug concentration in the target organ. In the intact skin the horny layer influences the drug behaviour due to its action as a penetration barrier on the one hand and its action as a drug reservoir on the other hand (10); the precise magnitude of each

function will determine the absorption rate of the substance into the skin and its concentration. Any disturbance of these functions, whether due to necessary clinical manipulation or, as is usually the case, due to pathological alterations in skin function, will eventually lead to gross modifications in the penetration rate and tissue concentrations of the drug. In addition, diffusion experiments through the epidermis (6) have suggested that an increase in drug concentration in the ointment does not necessarily result in a corresponding increase in its tissue concentration. This has to be expected since the amount of drug which can be removed from the skin surface even after a prolonged application by far exceeds the penetrating quantity (9, 11).

In the present study, the correlation between cortisol concentration in ointments and the resulting drug concentration in the various skin layers was investigated. These studies were carried out in vitro on both normal skin and on skin from which the horny layer had been removed by stripping (2). In addition, in vivo studies were performed in which cortisol ointments were applied to the backs of volunteers under conditions of occlusion and the vasoconstriction was measured according to the procedure of McKenzie (5).

MATERIAL AND METHODS

Cortisol ointments containing 0.1%, 0.3%, 1% and 3% drug were prepared by mixing uniformly labelled ^3H -cortisol (220 mCi/mg, Amersham Buchler, Braunschweig) and unlabelled drug into aqueous wool wax alcohol cream (German Pharmacopoea DAB 7). The mixing proportions were such that in all cases 5 mg ointment contained approximately 1 μCi -labelled drug.

In vitro experiments were carried out on human mam-

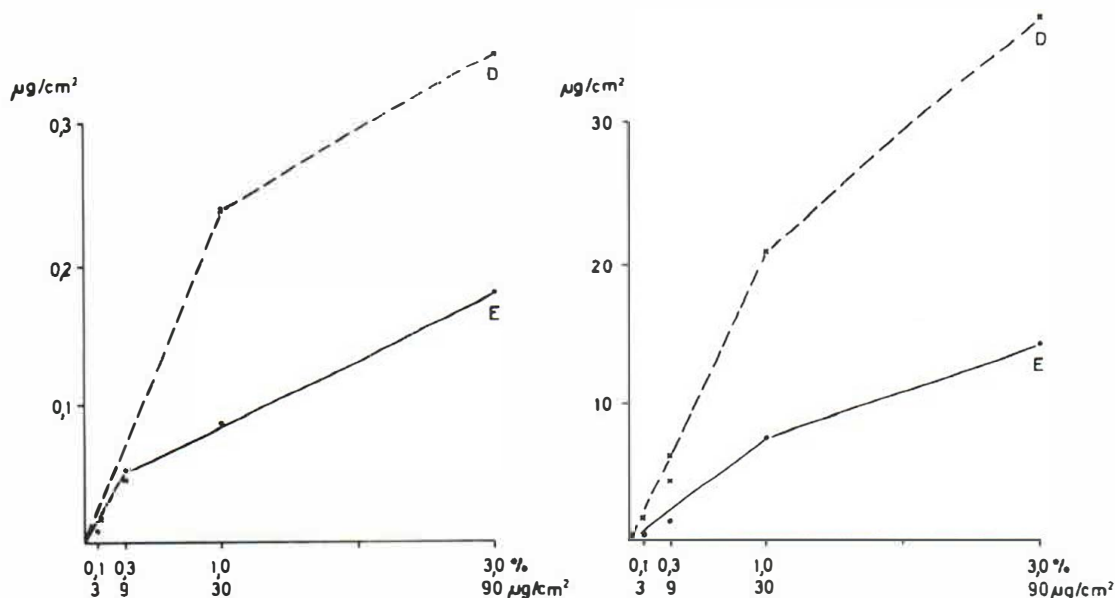


Fig. 1. Amount of drug (μg) in the respective tissue (E =epidermis, D =dermis) below 1 cm^2 skin surface in relation

to the proportions of drug in the ointment. *Left*: normal horny layer; *right*: horny layer removed.

mary skin. In each case, 25 mg ointment was applied to 7 cm^2 surface of one skin specimen. The ointment was uniformly rubbed in with a glass spatula and the skin was placed in a penetration chamber (9) for 90 minutes at a constant temperature of 32°C . The remainder on the spatula was weighed. The applied quantity (3 mg/cm^2) represents the amount which is normally applicable in therapy. Parallel experiments were carried out on another piece of skin from the same donor from which the horny layer had been removed by means of adhesive tape (TesaFilm) stripping (7). After 90 minutes, the remainder on the skin surface was removed by wiping the skin three times with dry cotton and the horny layer (when present) was removed by stripping as described earlier (3, 9, 11).

In parallel experiments, unlabelled ointments with the same base and concentrations of hydrocortisone (Cortisol) were investigated under occlusion in the vasoconstriction test according to McKenzie & Stoughton (4) and graded according to Moore-Robinson et al. (5). The

blanching caused by the vehicle without cortisol was termed "I" and the results with the cortisol ointments were corrected relative to this blanching.

RESULTS

1. The concentrations found in the epidermis and dermis increased with increasing concentrations of active substance in the applied ointment (Fig. 1; Tables I and IV).

2. The graphs of concentrations in the epidermis and dermis with intact and horny layer-free skin exhibit curves with two approximately linear regions (Fig. 1): (a) In the first segment (hydrocortisone applied at concentrations of up to 1%) the concentration in the tissue increased at roughly the

Table I. Amount of hydrocortisone ($\mu\text{g/cm}^2$ skin surface) penetrating into the tissue relative to concentration in vehicle

Percent concentration of hydrocortisone in vehicle	Horny layer (HL)	Epidermis		Corium		Whole skin	
		After HL removed	Intact HL	After HL removed	Intact HL	After HL removed	Intact HL
0.1	0.60	0.43	0.008	1.65	0.017	2.07	0.025
0.3	1.66	1.37	0.053	4.2	0.046	5.6	0.10
1	10.5	7.6	0.087	21.0	0.240	28.6	0.327
3	16.7	14.4	0.182	37.8	0.354	52.18	0.536

Table II. Increase in concentration in externum (doubling) and the resultant concentration increase in the epidermis (in each case in the linear segment of graph in Fig. 1)

	Intact horny layer				Horny layer removed			
Percent concentration increase in vehicle	0.1-0.3	0.1-0.2 ^a	1-3	1-2 ^a	0.3-1	0.3-0.6 ^a	1-3	1-2 ^a
Absolute increase of amount applied to skin ($\mu\text{g}/\text{cm}^2$)	3-9	3-6	30-90	30-60	9-30	9-18	30-90	30-60
Absolute increase of concentration in epidermis calculated on a specimen with 1 cm^2 surface ($\mu\text{g}/\text{cm}^2$)	0.008-0.053	0.016 ^a	0.087-0.182	0.048 ^a	1.37-7.6	2.25 ^a	7.6-14.4	3.4 ^a
Percent concentration in epidermis when doubling the concentration in the vehicle		85		56		100		45

^a Interpolated from Fig. 1.

same rate as the increase in the external concentration. A doubling of the concentration applied thus caused an increase in the skin concentration (epidermis and dermis) of 100% (Tables II, III). This is also true of the percentage increases after removal of the horny layer. (b) In the second segment of the curve (concentration increase from 0.3-3%), a doubling of the external concentration resulted in an increase in the hydrocortisone levels of only 20-40% in the dermis and 50% in the epidermis (Tables II, III).

3. At the lower external concentration levels (0.1-0.3%), the removal of the horny layer increased the epidermal concentrations by 40-50 times, and at higher concentration levels (0.3-3%) by 80-90 times (Tables I, IV).

4. Levels of hydrocortisone (cortisol) in the dermis were increased by a uniform 100 times,

following removal of the horny layer, at all external concentrations applied (Tables I, IV).

5. Vasoconstriction increased with increases in the drug concentration in the range 0.1-1%. However, higher concentrations did not induce a further effect on the circulatory system (Fig. 2, Table IV).

DISCUSSION

According to previous results no essential further increase in concentrations in the epidermis and dermis is seen after 100 min penetration time of hydrocortisone in vivo (9, 11).

The maximum of blanching, however, is reached within 12-16 h (4, 8). Thus it is interesting to note that there is a marked delay between the arrival of the hydrocortisone in the target tissue and the appearance of the pharmacological response. This

Table III. Increase in concentration in externum and the resultant concentration increase in the dermis (in each case in the linear segment of graph in Fig. 1)

	Intact horny layer				Horny layer removed			
Percent concentration increase in vehicle	0.1-0.3	0.1-0.2 ^a	1-3	1-2 ^a	0.3-1.0	0.3-0.6 ^a	1-3	1-2 ^a
Absolute increase of amount applied to skin ($\mu\text{g}/\text{cm}^2$)	3-9	3-6	30-90	30-60	9-30	9-18	30-90	30-60
Absolute increase in concentration in dermis calculated on a specimen with 1 cm^2 surface ($\mu\text{g}/\text{cm}^2$)	0.017-0.046	0.024 ^a	0.240-0.354	0.055 ^a	4.2-2.1	6.3 ^a	21.0-37.8	8.3 ^a
Percent concentration in dermis when doubling the concentration in the vehicle		105		23		105		40

^a Interpolated from Fig. 1.

Table IV. Concentration of hydrocortisone (μmolar) in the tissue (in vitro) and the effect of vasoconstriction relative to concentration in vehicle

HL=horny layer

Percent concentration of hydrocortisone in vehicle	Epidermis		Corium		Vasoconstriction (effect of vehicle=1)
	With HL	Without HL	With HL	Without HL	
0.1	1.35	73.4	0.44	42	1.05
0.3	9.2	235	1.2	162	1.28
1	15	1 209	6.16	537	1.67
3	31.4	2 480	9.1	966	1.66

demonstrates that in the vasoconstrictory reaction, it is not the transport of the drug to the target but the constrictory response itself which is the time-limiting process.

It has to be re-emphasized that because of the time-consuming procedure of these investigations, no repeated experiments can be performed for any one point in time and drug concentration so as to allow statistical evaluation. These earlier results (3, 9, 11) demonstrate, however, that the single data support one another in relation to the course of penetration process and the different concentrations.

The results presented here suggest that the vessels in the dermis react to a definite cortisol concentration. A higher drug concentration at the site of action does not cause any enhanced vasoconstriction (1000 min penetration time) and thus similar results with a fluorinated steroid as reported

by Christie & Moore-Robinson (1) are corroborated. It is interesting that the standard therapeutic dose for many years, a 1% hydrocortisone ointment, suffices for the establishment of the therapeutically effective concentration in the skin. A 6 μmolar hydrocortisone concentration (after a penetration time of 90 min only) is achieved in the dermis when using a 1% hydrocortisone ointment. A concentration increase from 1% to 3% in the ointment applied effects only a 50% increase in dermis concentration. In the epidermis a 100% concentration increase was found. However, only exponential concentration changes on the receptor side are capable of evoking discernible stepwise alterations in this pharmacological response (1, 5). Thus, this minor increase in concentration could not be expected to result in an increase in vasoconstriction.

It may be surmised that the concentration in-

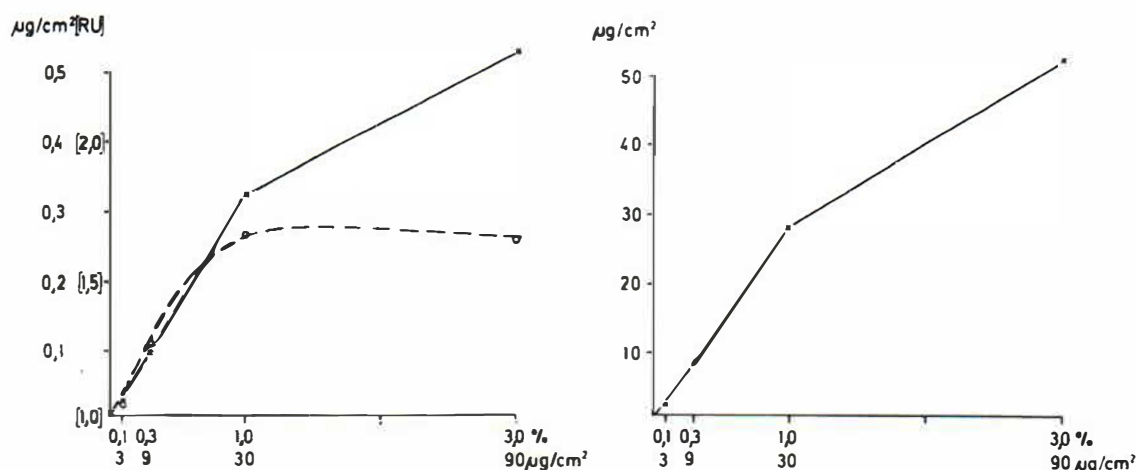


Fig. 2. Relation between drug concentration in the ointment, amount in whole skin (μg in a tissue segment with 1 cm^2 surface) and vasoconstriction. Vasoconstrictor effect of the vehicle itself=1 relative unit (RU). Left: normal horny layer; right: horny layer removed. —=concentration. ---=degree of vasoconstriction.

crease in vivo is actually lower, since the rate of further transport of drug into the circulatory system is increased when higher concentrations are applied externally (11). Similarly, the upper segment of the concentration curve of the intact skin (Fig. 2) is probably flatter and thus corresponds better to the vasoconstriction curve.

The clinical experience that no increase in effectiveness can be achieved with hydrocortisone preparations containing more than 1% drug, can be explained by these results showing that relevant concentration increases in the tissue are not possible. On the contrary, the therapeutic range of the ointment decreases as more substance is absorbed systemically for the same local effect.

If hydrocortisone cream is applied to skin free of horny layer, as is the case in injuries or in severe inflammations, a 0.1% preparation, for instance, yields the same tissue concentration as a 3% one in the case of intact skin. The therapeutic range of such preparations is therefore considerably narrowed in such pathological cases. Thus, investigations of vasoconstriction in the skin free of horny layer would appear necessary in order to ascertain the concentration present before application of further steroid ointment. Further investigations are required to ascertain to what extent a further transport through the vessel system lowers the concentration in the dermis. Higuchi's study (2) showing that doubling of the concentration causes only a maximal 40% increase in tissue level does not appear to have general validity, according to our findings. These theoretically obtained results are valid only at concentrations above 1% and for skin deprived of the horny layer. At lower concentrations (and this is true for most highly active synthetic corticosteroids) it is to be expected that doubling of the concentration of steroid applied externally leads to a doubling of the tissue concentrations. When the horny layer is removed, the epidermis takes up the same amount of drug as did the horny layer formerly (Table I). The barrier function of the horny layer, however, is lost completely.

REFERENCES

1. Christie, G. A. & Moore-Robinson, M.: Vehicle assessment, methodology and results. *Br J Derm* 82, Suppl. 6: 93, 1970.
2. Higuchi, I.: Physical chemical analysis of percutaneous absorption process from creams and ointments. *J Soc Cosmet Chem* 11: 85, 1960.
3. Kammerau, B., Zesch, A. & Schaefer, H.: Absolute concentrations of dithranol and triacetyl-dithranol in the skin layers after local treatment: in vivo investigations with four different types of pharmaceutical vehicles. *J Invest Dermatol* 64: 145, 1975.
4. McKenzie, A. W. & Stoughton, R. B.: Method for comparing percutaneous absorption of steroids. *Arch Dermatol* 86: 608, 1962.
5. Moore-Robinson, M. & Christie, G. A.: Vasoconstrictor activity of topical corticosteroids. Methodology and results. *Br J Dermatol* 82, Suppl. 6: 86, 1970.
6. Polano, M. K., Ponc, M., Smeenk, G. & Hendrikse, J. C. M.: Factors influencing the penetration of corticosteroids through the epidermis. *In Pharmacology and the Skin*. (W. Montagna, R. B. Stoughton, eds.), p. 325, Appleton-Century-Crofts New York 1972.
7. Pinkus, H.: Examination of the epidermis by the strip method of removing horny layers. *J Invest Dermatol* 16: 383, 1951.
8. Stoughton, R. B.: Vasoconstrictor activity and percutaneous absorption of glucocorticosteroids. *Arch Dermatol* 99: 753, 1969.
9. Zesch, A. & Schaefer, H.: Penetrationskinetik von radiomarkiertem Hydrocortison aus verschiedenen Salbengrundlagen in die menschliche Haut. I. In vitro. *Arch Derm Forsch* 246: 335, 1973.
10. Zesch, A., Schaefer, H. & Hoffmann, W.: Barriere- und Reservoirfunktion der einzelnen Hornschichtlagen der menschlichen Haut für lokal aufgetragene Arzneimittel. *Arch Derm Forsch* 246: 103, 1973.
11. Zesch, A. & Schaefer, H.: Penetrationskinetik von radiomarkiertem Hydrocortison aus verschiedenartigen Salbengrundlagen in die menschliche Haut. II. In vivo. *Arch Derm Forsch* 252: 245, 1975.

Received April 5, 1976

A. Zesch, M.D.
Department of Dermatology
The Free University of Berlin
Rudolf Virchow Hospital
Augustenburger Platz 1
D-1000 Berlin 65
FRG