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Development of Glucocorticosteroids with Enhanced Ratio between Topical and Systemic Effects

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A high potency at the application site and a low incidence of glucocorticoid side-effects form the desired profile of glucocorticosteroids for anti-inflammatory therapy. A new type of glucocorticosteroid 16,17-acetals with an improved topical/systemic activity ratio has been developed. Non-symmetric 16,17-acetal substitution increased the topical anti-inflammatory activity more than the systemic activity in rodents, whereas fluorine substitution in 9α- or 6α,9α-positions increased the systemic more than the topical potency. The non-fluorinated, non-symmetric 16α,17α-acetal budesonide reached the highest ratio, which was five to ten times better than that of the earlier known 16,17-acetonides such as triamcinolone acetonide, or that of the 17α-esters such as beclomethasone 17α,21-dipropionate.

Although budesonide and betamethasone 17α,21-dipropionate have the same topical anti-inflammatory potency, the latter decreased plasma and urinary cortisol levels significantly more when ointment preparations were compared in volunteers.

Budesonide is efficiently biotransformed in the liver to metabolites such as 6β-hydroxybudesonide and 16α-hydroxyprednisolone, which are 50–100 times less potent than the parent steroid. In homogenates of rat or human adult livers budesonide is biotransformed two to five times more rapidly than desonide and triamcinolone acetonide.

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Since their introduction 40 years ago glucocorticosteroids have been the most efficacious drugs for relieving inflammation. The endogenous hydrocortisone (Fig. 1) was the first to be used for treatment of skin diseases (1). The disappointment arising from the observation of severe adverse effects on long-term therapy of rheumatoid arthritis with cortisone stimulated structure-activity studies of glucocorticosteroids to separate anti-inflammatory potency from adverse effects.

Early research was concentrated on the improvement of the systemic anti-inflammatory activity and the lowering of unwanted side-effects such as sodium retention. The systemic activity of hydrocortisone was enhanced by insertion of an extra double bond in the 1,2-position or by introduction of a fluorine atom in the 9α-position (Fig. 1) resulting in prednisolone and 9α-fluorohydrocortisone, respectively. The latter substitution resulted in the highest potentiation but this was accompanied by a disturbance of the electrolyte balance. However, this was effectively counteracted by further substitution at the 16-position with an α-hydroxy, α-methyl or β-methyl group resulting in triamcinolone, dexamethasone and betamethasone, respectively (2). These studies gave no clear indication on separation of anti-inflammatory activity from adverse effects other than the disturbance of electrolyte balance.

Adrenal suppression, osteoporosis and decreased resistance to infection continued to be a problem. One way of minimizing adverse effects involves the topical application of steroids to the inflamed tissue, thus allowing smaller systemic concentrations of the hormone. The skin is perhaps the easiest target for such a therapy.

To make systemically potent glucocorticosteroids suitable for topical use on the skin, their lipophilicity must be increased. This is achieved by masking one or more of the hydrophilic hydroxyl groups in the 16α-, 17α- or 21-positions with a lipophilic group (3). Examples of such structural changes are the modifications of triamcinolone to triamcinolone acetonide and hydrocortisone to hydrocortisone 17α-butyrate (Locoid®) (Fig. 1). There are two types of parent glucocorticosteroids which can be modified for topical use, namely 16-methyl and 16α-hydroxy steroids such as dexamethasone, betamethasone and triamcinolone. The 16-methyl steroids are made lipophilic by esterification of the 17α- or the 17α- simultaneously with the 21-hydroxy groups (Fig. 1), e.g. betamethasone 17α-valerate (Betnovate®) and betamethasone 17α,21-dipropionate (Diproderm®) (3). The 16α-hydroxy steroids are made topically active by masking the 16α- and 17α-hydroxy groups to form a 16,17-acetonide, e.g. desonide, triamcinolone aceto-

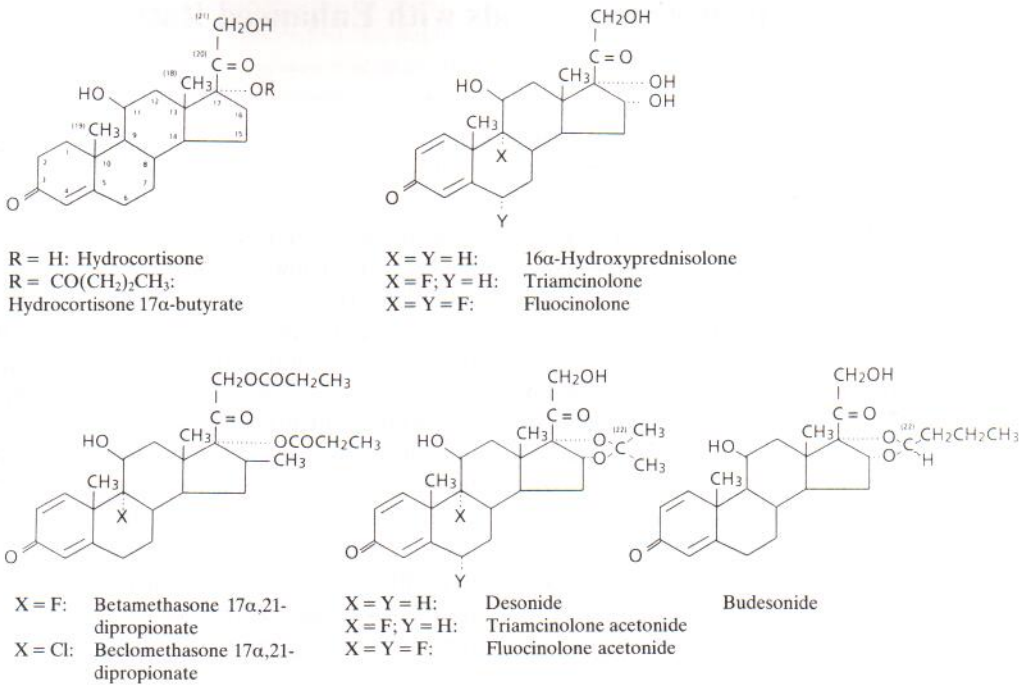


Fig. 1. Structures and generic names of the glucocorticosteroids discussed.

nide and fluocinolone acetonide (Fig. 1) (3).

The aim of the present investigation was to study how modifications in the structure of the 16,17-acetonide type of topical glucocorticosteroids would influence their effects and pharmacokinetics.

MATERIALS AND METHODS

Glucocorticosteroids

The structures and generic names of the 16α,17α-acetal glucocorticosteroids are shown in Fig. 1 and Table I. Two types of acetals were compared; the currently used 16,17-acetonides (acetal type A) and the new non-symmetric 16,17-acetals (acetal type B) (3–5).

Animals

The animal experiments were performed on young male Sprague Dawley rats weighing 90–100 g or male NMRI-mice weighing 20–25 g.

Human volunteers

Tests to determine the topical skin blanching potency of glucocorticosteroids were performed on the volar side of the arm.

The assessment of topical anti-inflammatory potency

The topical anti-inflammatory activity was measured as the potency to inhibit ear oedema formation in rats or mice using a model (6) which is a modification of the 'Tonelli test' (7). The glucocorticosteroids were applied topically as acetone solutions (20 μl/ear side in rats and 10 μl/ear side in mice) 16 hours before oedema induction. The topical 'blanching' potency was determined after the application of ethanol solutions of glucocorticosteroids on human skin. After evaporation of the solvent, the area was occluded under plastic wrapping for 18 hours (8).

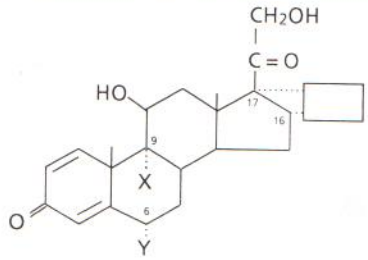
The assessment of systemic glucocorticoid potency

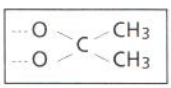
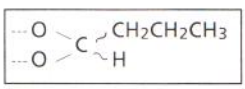
The systemic effects were assessed from the extent of thymus involution 4 days after epicutaneous application of the glucocorticosteroids in rats and 3 days after application in mice (6).

Relative binding affinity (RBA) for the glucocorticosteroid receptor

The binding affinity of the studied steroids for the glucocorticosteroid receptor of rat skeletal muscle was determined in relation to the affinity of dexamethasone (9).

Table I. *Topical anti-inflammatory activity measured as potency to inhibit rat ear oedema and systemic activity as thymus involution after epicutaneous application to rats. The activities are expressed in potency relative to budesonide. The ratio topical/systemic activity is calculated as the quotient between the relative potencies.*



Fluorine substitution in the 6- and 9-positions		Acetal in the 16,17-position Type A		Type B			
							
X	Y	Topical activity	Systemic activity	Topical activity	Systemic activity	Topical/ systemic activity	Generic name
H	H			1	1	1	Budesonide
F	H			2.2	2.5	0.88	S-1298
F	F			2.5	5.4	0.46	S-1314
H	H	0.2	2.2			0.11	Desonide
F	H	0.3	5.3			0.05	Triamcinolone acetonide
F	F	0.7	8.5			0.08	Fluocinolone acetonide

Biotransformation

The biotransformation has been studied extensively in vitro by incubation of tritium labelled glucocorticosteroids with the 9,000 g supernatant fraction of skin and liver from rat and man (10).

Effect on the hypothalamic-pituitary-adrenocortical (HPA) axis

Plasma and urine cortisol levels were assessed after treatment of volunteers on three consecutive nights with ointment preparations of Locoid® 0.1%, Diproderm® 0.05% and Preferid® 0.025%, the latter two equipotent preparations (11).

RESULTS

Topical anti-inflammatory potencies

The inhibition of ear oedema in rats by the 16α,17α-acetal substituted glucocorticosteroids is shown in Table I. The results indicate that it is more important to change the acetal substituent (from type A to type B) than to introduce fluorine atoms in the 6- and 9-positions in the steroid ring skeleton to achieve high topical anti-inflammatory potency.

The type B acetals were at least five times more potent than the corresponding type A compounds in inducing skin blanching and the change of the acetal type was more important than fluorine substitution in the steroid ring skeleton. Accordingly, there was a close correlation between the results obtained in the rat ear oedema model and the skin blanching test (6).

RBA for the glucocorticosteroid receptor

The RBA of the type B 16,17-acetal budesonide for the glucocorticosteroid receptor is approximately two, eight and 200 times higher than that of triamcinolone acetonide, dexamethasone and hydrocortisone, respectively (9). The correlation between RBA and topical anti-inflammatory potency in the rat ear oedema model (Fig. 2) is close, supporting the view that the topical activity at the application site is greatly dependent on the affinity for the glucocorticoster-

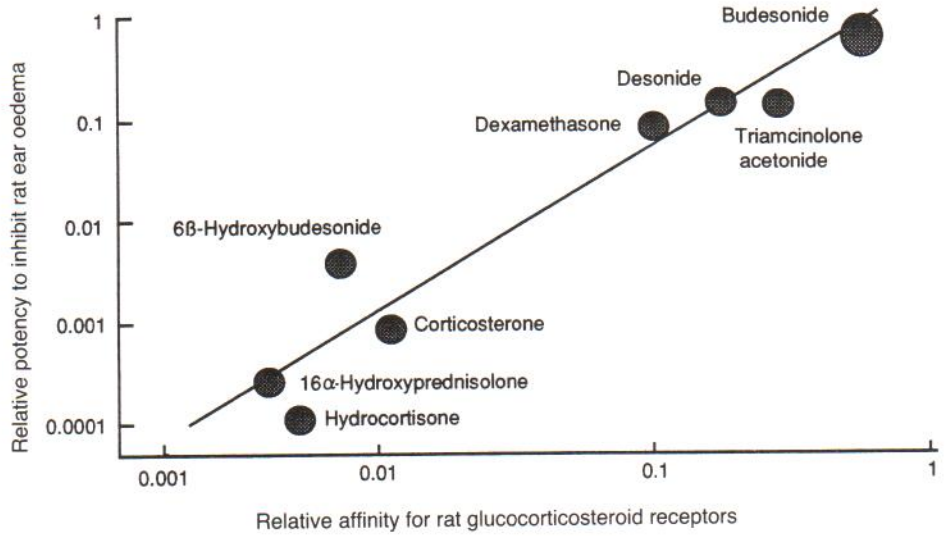


Fig. 2. Correlation between RBA for rat glucocorticosteroid receptors (determined in vitro) and topical anti-inflammatory potency in the rat ear oedema model. (Adapted from (9).)

oid receptor. On the other hand, the correlation between RBA and the thymus involution is low (9). This indicates that the pharmacokinetic properties of the individual glucocorticosteroids also contribute to the systemic effects.

Systemic glucocorticoid potency in rats

By increasing the topical doses it was also possible to induce systemic effects. The type B acetals had the same or lower systemic potencies than the corresponding type A glucocorticosteroids (Table I). Ring skeleton fluorination greatly enhanced systemic potency. For example, fluocinolone acetonide has eight to nine times the systemic activity of budesonide.

The relation between topical anti-inflammatory and systemic potencies in rats

The selectivity for topical activity can be expressed as a ratio between the potencies of topical and systemic activity (Table I). The new type B acetals attain approximately a tenfold better ratio than the earlier available 16α,17α-acetonides (type A). The highest ratio was reached for budesonide which accordingly is a more selective glucocorticosteroid for topical therapy than the 16α,17α-acetonides desonide, triamcinolone acetonide and fluocinolone acetonide (Table I).

Table II. *Potency of some glucocorticosteroids used in skin therapy compared with budesonide to induce skin blanching in man (8).*

Compound	ED_{50} (μg/ml)	Relative potency
Budesonide	0.9 (0.6– 1.2)	1
Betamethasone 17α,21-dipropionate	0.9 (0.6– 1.3)	1
Betamethasone 17α-valerate	1.8 (1.3– 2.5)	0.5
Fluocinolone acetonide	2.2 (1.5– 3.3)	0.4
Hydrocortisone 17α-butyrate	7.8 (4.8–12.3)	0.1
Desonide	10.2 (6.7–15.6)	0.1

Topical selectivity of budesonide in relation to the 16β-methyl 17α- and 17α,21-ester steroids

The topical selectivity of budesonide has been compared with those of betamethasone 17α-valerate and beclomethasone 17α,21-dipropionate using similar methods as described above. Assessment was carried out in mice as the latter two steroids had no topical anti-inflammatory effects in rats (12). Betamethasone 17α-valerate (Betnovate®) and beclomethasone 17α,21-dipropionate represent the other main type of topically active glucocorticosteroids (3). Beclomethasone 17α,21-dipropionate is the analog of betamethasone 17α,21-dipropionate (Diproderm®) in which the 9α-fluorine atom has been replaced by a 9α-chlorine atom (Fig. 1). Beclomethasone 17α,21-dipropionate (Becotide®) has been used preferably in asthma and rhinitis therapy. The activity of betamethasone 17α-valerate (unpublished results) and beclomethasone 17α,21-dipropionate (13) to inhibit ear oedema formation was approximately half that of budesonide, which is consistent with the skin blanching potency in man (Table II) (14). The ability to induce thymus involution in mice after topical application was three to four times higher for beclomethasone 17α,21-dipropionate than for budesonide. Betamethasone 17α-valerate and budesonide had a similar potency. Thus, budesonide also attained a twofold better ratio between the topical and systemic activities than betamethasone 17α-valerate (unpublished results) and a tenfold better ratio than beclomethasone 17α,21-dipropionate (13).

Human skin blanching test

The ability of budesonide to induce skin blanching in man was compared with some commonly used glucocorticosteroids (Table II). Budesonide and betamethasone 17α,21-dipropionate (Diproderm®) are of equal potency. Betamethasone 17α-valerate (Betnovate®) and fluocinolone acetonide (Synalar®) show medium potency, whereas hydrocortisone 17α-butyrate (Locoid®) and desonide (Apolar®) were the least active substances in this test (8).

Biotransformation in skin

Incubation experiments in human and rat skin homogenates with budesonide, hydrocortisone and triamcinolone acetonide showed that little or no biotransformation occurred (10).

Biotransformation in liver tissue

The biotransformation rate of budesonide, desonide and 16α-hydroxyprednisolone has been compared in vitro in human and rat liver preparations (15). Budesonide was biotransformed six times more rapidly than desonide, whereas 16α-hydroxyprednisolone was biotransformed very slowly, if at all, in human liver (Fig. 3). The rank order was the same in rat liver. The biotransformation rate of budesonide in human liver was also twice and six times as high as that of triamcinolone acetonide and hydrocortisone, respectively (Fig. 4) (10). In rat liver the order was hydrocortisone > budesonide > triamcinolone acetonide, and the biotransformation rate of budesonide was five times more rapid than that of triamcinolone acetonide (10).

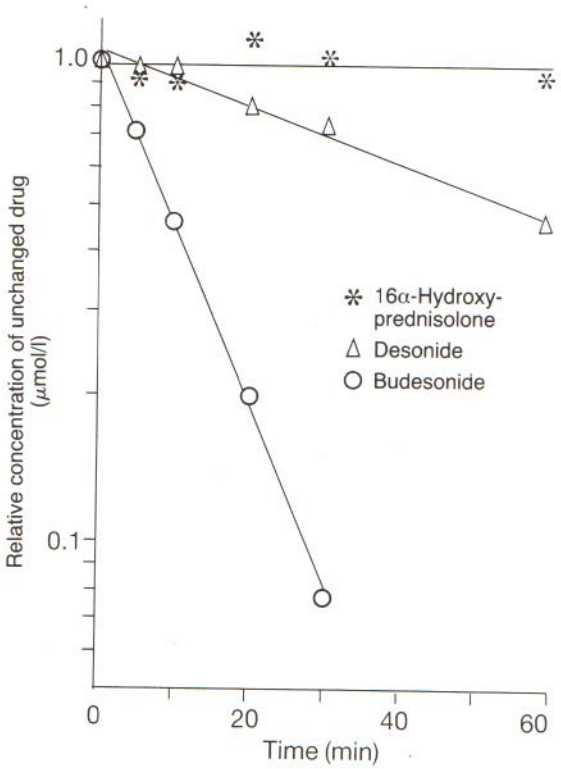


Fig. 3. The influence of 16,17-acetal substitution on the in vitro biotransformation rate in human liver preparation (pooled livers from six individuals) (16).

Effect on the HPA-axis

The systemic side-effects of budesonide, measured as an inhibition of the function of the HPA-axis, were compared with those of the ester structures hydrocortisone 17α-butyrate and betamethasone 17α,21-dipropionate in volunteers (11). Budesonide and hydrocortisone 17α-butyrate were not significantly dif-

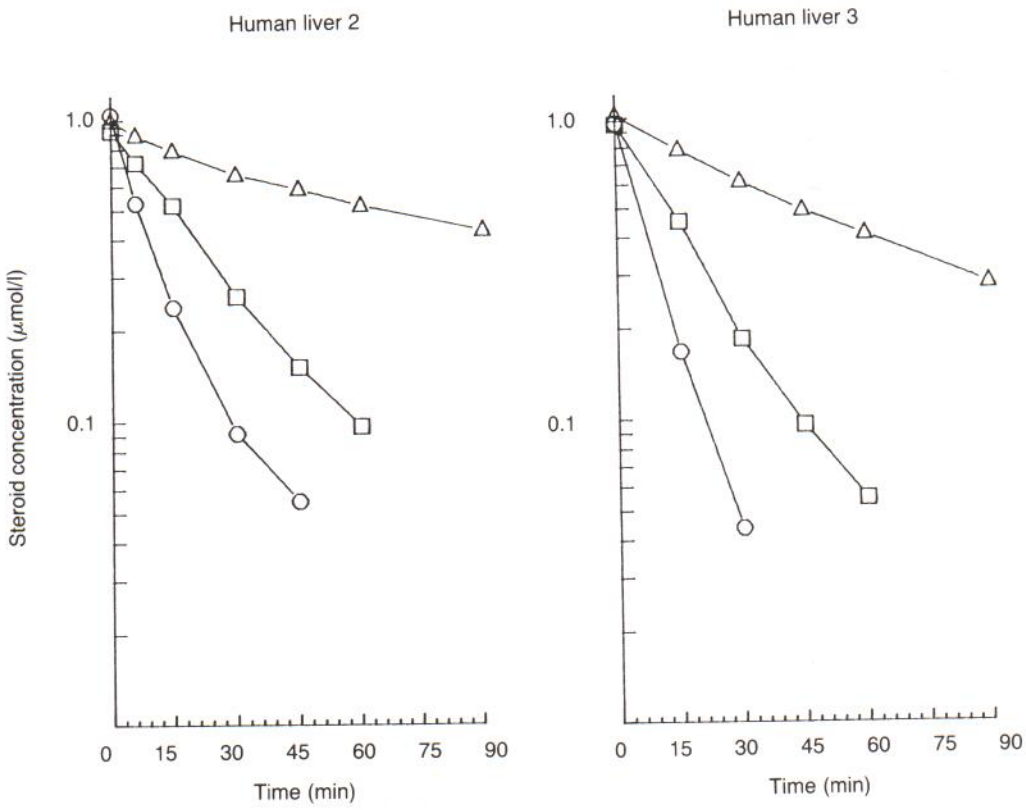


Fig. 4. Biotransformation rates of budesonide (○), triamcinolone acetonide (□) and hydrocortisone (△) in human liver preparation. The decrease in concentration of unchanged steroid in two out of five livers is shown. Initial glucocorticosteroid concentration was 10^{-6} mol/l (10).

ferent at any time and they caused at the most a 50% suppression of the plasma cortisol level. Betamethasone 17α,21-dipropionate reduced the plasma cortisol level at the most by 96% (Fig. 5). Six out of nine volunteers had to be withdrawn after two nights of treatment with the latter compound due to too low cortisol levels. These six subjects are shown separately in Fig. 5B. The variations in 24-hour urinary cortisol excretion show a similar behaviour as the plasma cortisol levels (11).

DISCUSSION

To increase the potency of glucocorticosteroids intended for systemic use, it has been common to reduce the biotransformation rate and the transcortin binding, e.g. triamcinolone, dexamethasone and betamethasone. Increased affinity for the glucocorticosteroid receptor may also be a contributory factor (9). The relative importance of each of these factors has not been assessed so far. Therefore, the aim of the present studies was to investigate how structural variations in the 16,17-acetal substituent as well as the fluorine substitutions in the 6- and 9-position of the steroid ring skeleton would influence the topical and systemic activities in relation to receptor affinity and biotransformation rate.

The results show that it is more important to optimize the 16,17-acetal substituent than to introduce fluorine atoms in the 6- and 9-positions if a potent topical anti-inflammatory glucocorticosteroid is desired. The highest activity was obtained when a hydrogen atom and a straight alkyl chain containing three carbons was substituted in the 22-position in the molecule (cf. budesonide in Fig. 1). Fluorine substitution in the ring skeleton, on the other hand, potentiated the systemic activity to a higher degree than the topical activity. Accordingly, budesonide reached a much better topical/systemic activity ratio

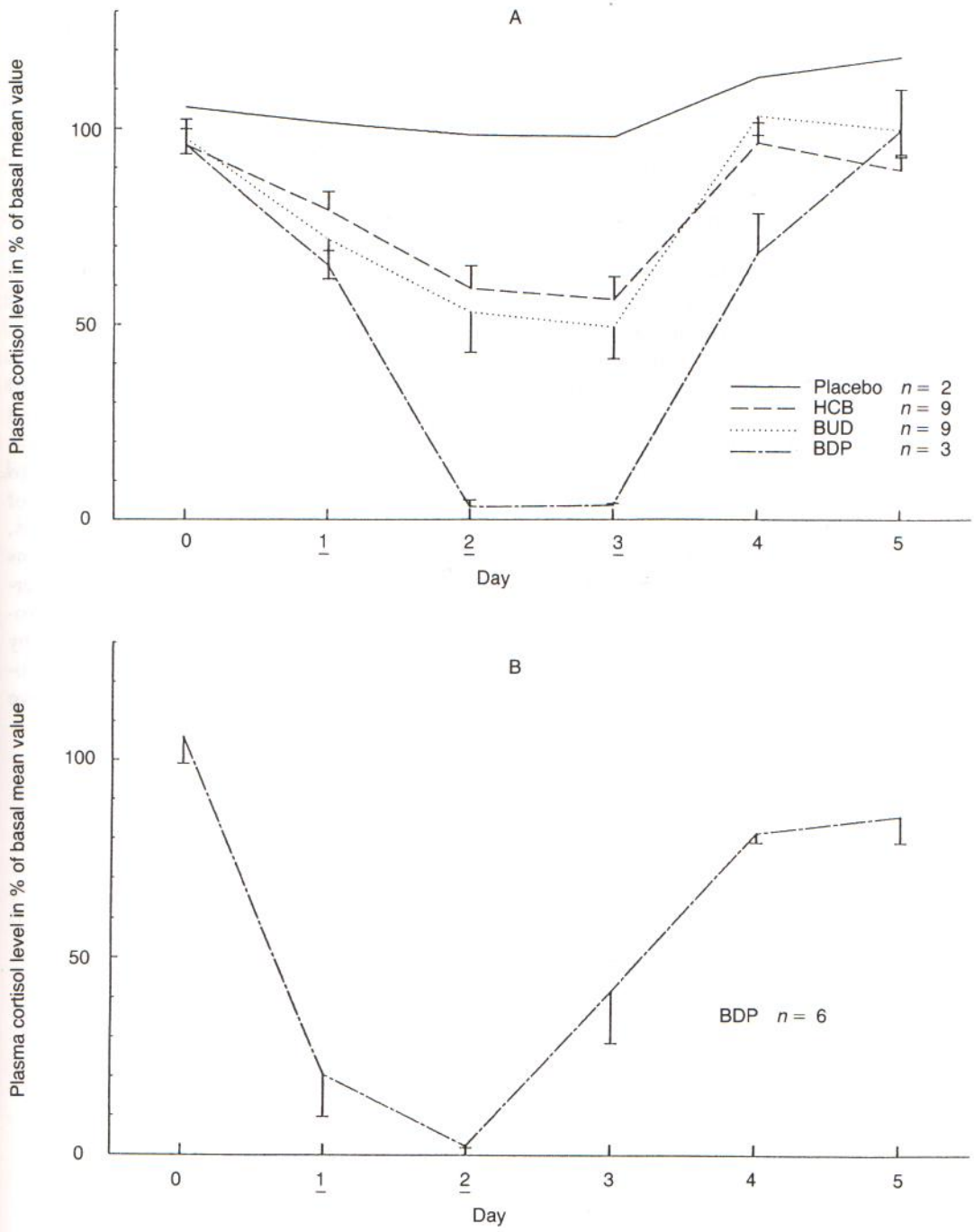


Fig. 5. Changes in plasma cortisol in volunteers of budesonide (BUD, Preferid®), hydrocortisone 17α-butyrate (HCB, Locoid®) and betamethasone 17α,21-dipropionate (BDP, Diproderm®) ointment preparations as percentage of basal mean value after three nights of occlusion (A) and after two nights of occlusion with Diproderm® (B). Starting values for plasma cortisol were taken and are marked in A and B with SEM values (day 0). Underlined days indicate treatment days (11).

halogenated 16,17-acetonides in rats or the 17α-ester compounds such as beclomethasone 17α,21-dipropionate and betamethasone 17α-valerate in mice (results not shown).

Budesonide is a very potent glucocorticosteroid at the site of application due to a high affinity for the receptor. The lack of biotransformation in the skin contributes to a high local concentration of the drug in the target organ.

When budesonide was given by systemic routes its anti-inflammatory potency was not greater than its other types of systemic glucocorticoid activities, e.g. thymus involution (4). This means that at the receptor level budesonide, like other glucocorticosteroids, cannot differentiate between anti-inflammatory and other types of glucocorticoid effects (17). Thus, inactivation by metabolic transformation in the liver after absorption from the sites of deposition explains the low systemic activity of budesonide.

Budesonide is oxidized or reduced by the liver enzymes into several metabolites. The oxidized products 6β-hydroxybudesonide and 16α-hydroxyprednisolone are the major metabolites in man (Fig. 6) (18–20). Both have negligible affinity for the glucocorticosteroid receptors (Fig. 2). The latter metabolite is formed by cleavage of the 16,17-acetal after incorporation of oxygen at the 22-carbon in the oxidation step (21). The 16,17-acetonides do not undergo this type of metabolic cleavage since they provide no site of oxygenation at the 22-carbon, probably due to the absence of a hydrogen atom at this position. Thus, the metabolism of budesonide follows two pathways, whereas the 16,17-acetonides follow only one. This contributes to the effective inactivation and favourable local/systemic activity ratio of budesonide.

To judge the clinical value of the selectivity obtained with budesonide, it was also necessary to make a comparison with the selectivity of the 17α- and 17α,21-esters, e.g. hydrocortisone 17α-butyrate and betamethasone 17α,21-dipropionate (11). Comparative studies of systemic side-effects are difficult to perform in patients due to gradual improvement of the skin barrier function during the healing process, and also due to intra- and inter-individual variations of the lesions. Therefore, the investigation was performed in healthy volunteers in whom a more reproducible defect of skin barrier function was induced by the standard occlusion technique. Although budesonide and betamethasone 17α,21-dipropionate have

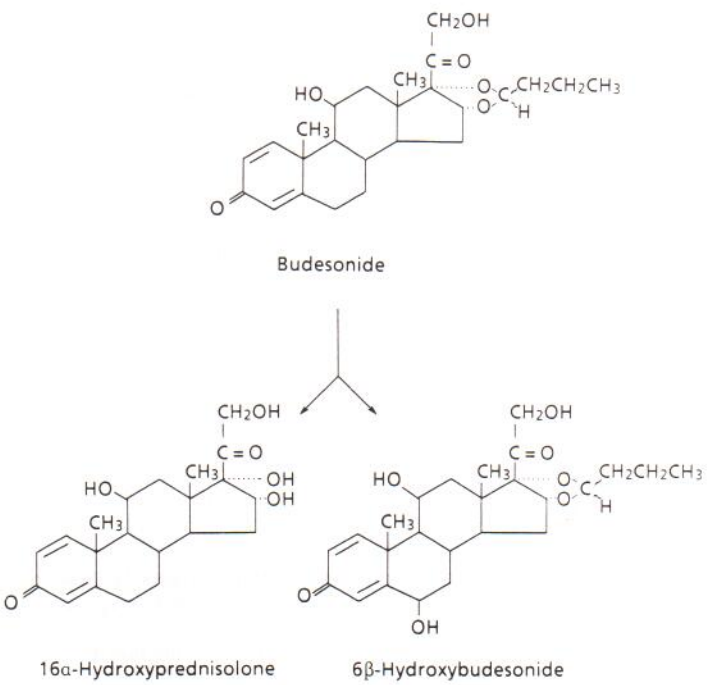


Fig. 6. Structures of 16α-hydroxyprednisolone and 6β-hydroxybudesonide, the two major metabolites of budesonide.

the same topical anti-inflammatory potency (8) the latter caused a significantly greater suppression of both plasma and urinary cortisol levels. No significant difference was found between budesonide and hydrocortisone 17α-butyrate (11) despite the large difference in topical anti-inflammatory potency (8).

In conclusion, the non-symmetrical (type B) 16,17-acetals (e.g. budesonide) are very potent topical glucocorticosteroids. Due to the inactivation by rapid and efficient liver metabolism they are more selective for the topical treatment of skin sites than the currently used 16,17-acetonides, and the 16β-methyl 17α-esters such as betamethasone 17α-valerate and betamethasone 17α,21-dipropionate.

REFERENCES

1. Sulzberger MB, Witten VH. The effect of topically applied compound F in selected dermatoses. J Invest Dermatol 1952; 19: 101–102.
2. Sarrett LH, Patchett AA, Steelman SL. The effects of structural alteration on the anti-inflammatory properties of hydrocortisone. In Jucker E, ed.: Progress in Drug Research. Basel: Birkhäuser Verlag, 1963; 5: 13–153.
3. Phillipps GH. Locally active corticosteroids: structure-activity relationships. In Wilson L, Marks R, eds.: Mechanisms of topical corticosteroid activity. Edinburgh: Churchill Livingstone, 1976; 1–18.
4. Thalén A, Brattsand R. Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with high local activity. Arzneim-Forsch/ Drug Res 1979; 29: 1687–1690.
5. Thalén A, Brattsand R, Gruvstad E. Synthesis and pharmacological properties of some 16α,17α-acetals of 16α-hydroxyhydrocortisone, 16α-hydroxyprednisolone and fluorinated 16α-hydroxyprednisolones. Acta Pharm Suecica 1984; 21: 109–124.
6. Brattsand R, Thalén A, Roempke K, Källström L, Gruvstad E. Influence of 16α,17α-acetal substitution and steroid nucleus fluorination on the topical to systemic activity ratio of glucocorticoids. J Steroid Biochem 1982; 16: 779–786.
7. Tonelli G, Thibault L, Ringler I. A bioassay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied corticoids. Endocrinology 1965; 77: 625–634.
8. Gruvstad E, Bengtsson B. A comparison of a new steroid budesonide, with other topical corticosteroids in the vasoconstriction test. Drugs Exp Clin Res 1980; 6: 385–390.
9. Dahlberg E, Thalén A, Brattsand R, Gustafsson J-Å, Johansson U, Roempke K, Saartok T. Correlation between chemical structure, receptor binding and biological activity of some novel, highly active, 16α,17α-acetal substituted glucocorticoids. Mol Pharmacol 1984; 25: 70–78.
10. Andersson P, Edsbäcker S, Ryrfeldt Å, von Bahr C. In vitro biotransformation of glucocorticoids in liver and skin homogenate fraction from man, rat and hairless mouse. J Steroid Biochem 1982; 16: 787–795.
11. Scott M, Malmsten LÅ, Thelin I. Effect on plasma cortisol level and urinary cortisol excretion, in healthy volunteers, after application of three different topical steroid ointments under occlusion. Acta Derm Venereol (Stockh) 1981; 61: 543–546.
12. Axelsson B, Brattsand R, Andersson P, Ryrfeldt Å, Thalén A. Relation between the glucocorticosteroid effects of beclomethasone 17α,21-dipropionate, beclomethasone 17α-propionate and beclomethasone as studied in human, mouse and rat tissue. Respiration 1984; 46 (Suppl 1): 4–5.
13. Brattsand R, Thalén A, Roempke K, Källström L, Gruvstad E. Development of new glucocorticosteroids with a very high ratio between topical and systemic activities. Eur J Respir Dis 1982; 63 (Suppl 122): 62–73.
14. Johansson S-Å, Andersson K-E, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid potencies of budesonide and beclomethasone dipropionate in man. Eur J Clin Pharmacol 1982; 22: 523–529.
15. Andersson PH, Lihné M, Thalén A, Ryrfeldt Å. Effect of structural alterations on the biotransformation rate of glucocorticosteroids in rat and human liver. Xenobiotica 1987; 17: 35–44.
16. Andersson P. Structural aspects on the metabolism of glucocorticosteroids. Thesis, Lund University 1986.
17. Feldman D, Funder J, Loose D. Is the glucocorticoid receptor identical in various target organs? J Steroid Biochem 1978; 9: 141–145.
18. Edsbäcker S, Jönsson S, Lindberg C, Ryrfeldt Å, Thalén A. Metabolic pathways of the topical glucocorticoid budesonide in man. Drug Metab Dispos 1983: 6: 590–596.
19. Edsbäcker S. Studies on the metabolic fate and human pharmacokinetics of budesonide. Thesis, Lund University 1986.
20. Edsbäcker S, Andersson PH, Lindberg C, Paulson J, Ryrfeldt Å, Thalén A. Liver metabolism of budesonide in rat, mouse, and man. Comparative aspects. Drug Metab Dispos 1987; 15: 403–411.
21. Edsbäcker S, Andersson PH, Lindberg C, Ryrfeldt Å, Thalén A. Metabolic acetal splitting of budesonide. A novel inactivation pathway for topical glucocorticoids. Drug Metab Dispos 1987; 15: 412–417.

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Discussion

CHAIRMAN: PROF. V.K. HAVU

Prof. V.K. Havu (Finland): Dr Murray, do you think that new steroids are needed? There are plenty of very effective steroids already available.

Dr J.R. Murray (UK): That is not only an interesting question but also difficult to answer, except of course if it is considered in relationship to Dr Brattsand's paper regarding the metabolic degradation of corticosteroids. If we look intellectually at Wilbur Wright's machine in which he first flew, and compare it with a Concorde, we may argue that perhaps a Concorde is not necessary — but nevertheless it is there and it does serve some purpose, albeit perhaps only the transportation of wealthier people. The differences between those two aircraft were accomplished only by many small steps in between. Each step, however small, was important because it represented an advancement. The same stepwise development applies to therapeutic medicine.

There is another factor. I am basically a first-line physician, a general practitioner (GP), with a considerable interest in dermatology. It is the GP who is the main prescriber of topical corticosteroids in Western Europe, and it is the GP who needs steroids with a high ratio between topical activity and adverse reactions — because he is less likely to have the control over patients that physicians in hospital practices will certainly have.

One of the policies that Gist-brocades itself has relied on is the sort of three-phase concept, in which hydrocortisone is always used in the initial phase. Treatment is carried out with Locoid® (hydrocortisone 17-butyrate) if the lesion requires a steroid of medium potency. Another hydrocortisone derivative for a potent steroid is used only if the skin lesion is refractory to the mild as well as to the medium-potent steroid.

To answer your question, yes, I think there is a considerable need for new steroids, certainly one that may be just as useful to your GP colleagues as it is to myself.

Prof. Havu: Dr Kragballe, is the correlation between receptor binding and potency of steroids sufficient to explain potency differences of steroids? Is it simply a question of affinity to receptors?

Dr K. Kragballe (Denmark): According to studies by Ponec in 1980, receptors for steroids have been demonstrated to exist both in human epidermis and in fibroblasts. Binding of the steroid to the receptor was shown to be correlated with the capacity of the steroid to inhibit the proliferation of fibroblasts.

To answer your question, we can certainly agree that at least in some systems there is a correlation between receptor binding and the effect on cell function.

Prof. Havu: Are the receptors present in all tissues?

Dr Kragballe: Yes, I would say in most tissues; there are receptors on all normal cells of the body, and they all respond to glucocorticosteroids.

Prof. Havu: The mechanism of action of corticosteroids covers a vast and complicated area. You told us something about lipocortins. They explain many of the actions of steroids. Are there steroid effects which cannot be explained by the mediation of the lipocortins?

Dr Kragballe: Lipocortin is only one example of a protein which is affected by steroids. As I mentioned, interleukin-1 (IL-1) and possibly many other mediators are also affected by them. It was just an example of an important protein. For example, the effect on leukocyte chemotaxis is indirectly also dependent upon lipocortin because its chemotactic activity which is determined by phospholipase A2 is mediated by lipocortins. I think these are important.

Question from the audience: How do the steroids affect eicosanoids, prostaglandins and leukotrienes?

Dr Kragballe: Eicosanoids, prostaglandins and leukotrienes are derivatives of arachidonic acid; in other words, arachidonic acid is their precursor. The corticosteroids affect the formation of these compounds by modulating the release of this precursor, arachidonic acid. This means that by modifying the release of arachidonic acid from phospholipids the steroids will also affect the formation of other less related

compounds. In addition to modifying the formation of these compounds, the steroids will also inhibit the sensitivity of certain tissues to these mediators. Corticosteroids have therefore a dual effect; it is not an effect only on the formation but also on the sensitivity of the tissues to these mediators.

Dr J. De Bersaques (Belgium): It has been stated that all the effects of steroids are due to one receptor, and that it is the same receptor for all steroids. Are the effects produced through this receptor identical for each steroid once the steroid has bound to the receptor?

Dr Kragballe: It is not a question of a common receptor. There are specific receptors for each type of tissue, and in addition there are only certain genes in one cell type that are affected by this activated receptor complex. The set of genes that are modified by steroids are different from tissue to tissue, so there is no question of an effect on the same receptors or the same genes in a different tissue.

Dr De Bersaques: Considering your statement, my question is now whether different genes in one tissue are influenced if the steroid is changed? [*Dr Kragballe:* What do you mean by changing the steroid?] If another steroid is given to the same receptor, will this affect other genes, or will some other genes possibly be spared? In other words, if another glucocorticoid is given which binds to the receptor, will another group of genes be affected?

Dr Kragballe: I am not aware that this has been studied, but other members of the Panel might have more experience on this matter.

Dr R. Brattsand (Sweden): I think a clear answer cannot be given. Empirically, I do not know of any glucocorticosteroid which has such a differentiating effect on the receptor but, theoretically, I want to stress the fact that glucocorticosteroid receptors are composed of three different parts, of which the glucocorticosteroid site is one. When that part is triggered, the DNA binding site is opened, so that the DNA binding part of the receptor can fuse with DNA. It is complicated because the receptor has to be potentiated further by some other factor, and that could be on the part of the molecule where the steroid is situated. It is therefore this enhancing factor which induces the true promotion of the gene.

In this very complicated process there could be some minor differences because of the differently structured glucocorticosteroids, but much more time is needed to determine that explicitly.

Dr H.L. Muston (UK): If the effects of steroids are mediated only by means of this receptor, does it not follow that it would be impossible to differentiate between anti-mitotic, anti-synthetic and anti-inflammatory effects? If that is the case, if we cannot distinguish between different corticosteroids, does it not also follow that the only advance possible would be through manipulation with pharmacokinetics, so that the advances will be in terms of how the compound behaves in the body generally?

Dr Brattsand: I think I can agree with that. We can indeed not only change potency but also pharmacokinetics.

Dr Muston: Would it not follow that if it is impossible to distinguish between the wanted anti-inflammatory effects and the anti-mitotic and anti-synthetic effects, there is no point in worrying about the potency of new steroid molecules, and that we should really be looking at steroids in terms of their behaviour once they get into the body, which means their distribution and systemic side-effects?

Dr G.E. Piérard (Belgium): I do not think this will be the case. All different tissue and cell types do not respond at the same speed. In the epidermis, Langerhans cells are the first cells to respond, followed by keratinocytes, and subsequently there is the interrelation between keratinocytes and melanocytes. In the dermis, the dendrocytes respond first, and then other elements are affected which become manifest by characteristic side-effects. By manipulating the pharmacokinetics it would perhaps be possible to modify the metabolic activity of some cells and not that of others.

It seems, at least to me, that indeed all the cells that are involved in the immune response are more sensitive than other cells. Perhaps one day we may be able to find a good corticosteroid which would have an anti-inflammatory effect without any significant consequences for the structure of the skin.

Dr J. White (UK): Could the Panel explain the mechanism of tachyphylaxis? Secondly, are there differences between the various steroids, so that we could

possibly use them in a different intermittent way, and thereby get greater effect from them?

Prof. Havu: The phenomenon of tachyphylaxis means that the steroid first appears to cause vasoconstriction and decreased DNA synthesis but, as the days go by and treatment continues, vasoconstriction disappear or decrease while DNA synthesis starts increasing again even when steroid is present. The question is what happens, and why?

Dr Brattsand: We know that there is a downregulation. When studied actively in cells, we can see that one of the protein's messenger RNAs, which is downregulated within a few hours, is the glucocorticosteroid receptor itself.

That has been studied in vitro by Oikarinen and co-workers in Finland in cultured tissue fibroblasts. They cultured for 7 or 9 days, and reported a very strong downregulation of the receptors. There also seems to be an escape from the downregulation. At least, J.-A. Gustafson's group at the Karolinska Institute reported that there is an escape when the receptor is downregulated to about 40%. After that it is impossible to get any more downregulation. This may be one of the mechanisms of tachyphylaxis.

A further possibility — but this is more in the way of speculation — is that if an inflammation is present, there may be changes in the receptors by proteolytic enzymes. I have, however, no sound proof of that.

The tachyphylaxis problem does not occur in asthma inhalation, which seems strange, but it could have been due to the pulse exposure used. Inhalation is carried out twice a day, with a dose-free period between the inhalations. In rheumatoid arthritis it is a well-known fact that glucocorticosteroids help for some months, but subsequently the effect will decrease.

Dr Murray: It is an interesting question from the practical point of view because of course sometimes we GPs make use of tachyphylaxis in an odd sort of way. If I refer a patient to you because he is no longer responding to my therapy, and you change it to something wonderful, I do not tell the patient that you are a better doctor. I explain about tachyphylaxis and that it is the reason why treatment has been changed and become effective.

Dr A. Walker (UK): Are there any good studies to show that *when* we apply steroids to the skin, for example, when the skin has just been made warm by having a bath, or when the skin is cold, this will significantly affect the amount of absorption or indeed the efficacy of treatment?

Dr P.J. August (UK): I can answer that only indirectly. We measured some blood levels of Locoid® under various conditions, one of which was after people had been exercised and their skin made hot and sweaty, and of course the dermal blood flow had also been increased. The hydrocortisone butyrate level in the blood went up three- or fourfold. I suspect, therefore, that if the homeostasis of the skin is disturbed more steroid goes in.

I do not know whether this fact can be used therapeutically. On the whole, we prefer our patients with skin diseases not to exercise too much. Of course, one of the main purposes of admission to hospital is to avoid just that.

Dr Walker: It might explain, however, why sometimes steroid treatment does not appear to be effective. If it is put on when the skin is very cold, and therefore presumably vasoconstricted, it is not absorbed so well.

Dr August: Perhaps it has some relevance, say, to the hands, which are usually colder. I do not think we have ever considered it seriously before.

Prof. Havu: Dr Thalén, how would you explain the fact that de-acetylation or cleaving of the acetyl group from budesonide is more effective than, say, the same processes in corresponding prednisolone 16,17-acetonides?

Dr A. Thalén (Sweden): I think it is a matter of steric hindrance in the molecule, so that cytochrome P450 enzymes cannot reach the acetonide side chains of the 16,17-carbon atoms.

Question from the audience: Dr Ashworth, I very much enjoyed your paper. Could you give some idea about the dynamics of the processes? I am not quite clear about how long the steroid has to be on before the Langerhans cells all disappear, and also about the disaggregation and stimulation experiments.

Dr J. Ashworth (UK): The first experiment I did with different strength steroids was to look for an alternative way of classifying them simply. We initially as-

sessed the effect on six different strength steroids after one application. These preliminary experiments showed that a single application of most corticosteroids reduced Langerhans cell numbers. The assessment took place 24 hours after application.

Although five of the six different strength steroids reduced Langerhans cell numbers, there was no statistically significant difference between these five and hydrocortisone. That is why in the following assessments twice a day for 1 week was arbitrarily chosen. In other words, 24 hours after a single application there is some effect on Langerhans cell numbers and, one would assume, therefore some effect on immune function. Langerhans cell numbers seem to return to normal within about 14–21 days after stopping the application of topical corticosteroids.

With regard to function, we can observe that psoralen and ultraviolet-A (PUVA) therapy has similar effects on skin immunity because it decreases the allo-antigen presenting capacity of skin. If immune function is measured by allergic contact dermatitis reactions there is a return to normal about 3 weeks after stopping PUVA therapy. I guess that PUVA treatment and steroids have the same type of immunosuppression, but I have no data to prove it.

I think the effect starts almost immediately, and recovery takes about 3 weeks.

Prof. Havu: You suggested that the immune function tests which you presented could be used as an alternative to the vasoconstrictor test in screening steroids. However, your tests are very complicated and time-consuming. Are they a realistic alternative?

Dr Ashworth: The vasoconstrictor assay should be accepted for what it is, which is a very crude way of measuring an effect of a topically applied corticosteroid. It is also a reasonable screening procedure if you are considering very preliminary modes of screening of topical corticosteroids. I have great difficulty in believing that the anti-inflammatory effect of a topical corticosteroid is primarily upon blood vessel smooth muscle, which is what causes vasoconstriction.

As has been mentioned before, it is the GP who presumably prescribes the vast majority of topical steroid preparations that patients use. If we want to give them the best possible information in order to allow them to decide what they want to prescribe, it makes more sense to measure a function of steroids which is presumably much more directly relevant to their anti-inflammatory mechanisms. In general, most of the functional assays are expensive and time-consuming. I would not recommend using them as a primary screening procedure.

However, the measurement of their effect upon Langerhans cell markers is very simple, and lends itself easily to statistical analysis. Many different steroids may be compared together in the same individual, if desired, by using the skin of the back, for example. I do not think that is an unreasonable alternative.

The other point, of course, is that if we continue to use a vasoconstrictor assay, which is now 27 years old, and just accept it as a measurement of steroid efficacy, it does not help our understanding of what steroids do. It was asked earlier whether it is really true that the anti-inflammatory actions of steroids on the skin are not understood. I agree with that. We know that they do various things to IL-1 and so on, but what steroids do to the skin is not understood. I am simply trying to encourage people to look at the effects of steroids much more scientifically.

Dr Murray: From the point of view of the pharmaceutical industry, obviously the vasoconstrictor assay is extremely useful as a sort of blunderbuss type of screen. One of the biggest problems with which we are faced — and, as a clinician, I sometimes find it rather confusing — is the problem between formulations, where there is the same glucocorticoid but different vasoconstrictor assay results with different vehicles. Perhaps having something more precise or standardized would help us to choose the most appropriate vehicle for that particular steroid.

Prof. Havu: Dr Piérard, you showed us some very interesting pictures of dendritic cells in the dermis. Can you explain what those cells are and what they are doing?

Dr Piérard: We do not know what dendritic cells are doing. They have been identified by the fact that they have several features in common with the monocyte macrophage system. Dendrocytes are known to be capable of phagocytosing many things. They are present in the superficial dermis and are known to participate in several disorders. Specifically, dermatofibromas have now been described as dendrocytomas because they consist of cells which are probably dendrocytes. They are also found in reasonable numbers in other skin disorders, mainly those related to inflammatory and immune disorders.

It is now thought that these cells play a certain role in the immune surveillance within the skin, particularly within the dermis. They are affected by corticosteroids.

Dr E. Sahan (UK): Do you think that the different experimental tests available at present for the estimation of potency of local steroids give any idea of their long-term side-effects, for example, skin atrophy?

Dr Piérard: That is difficult to answer. There are many methods by which this type of effect can be measured. For example, ultrasound is now used to measure the thickness of the skin, but when differences in skin thickness occur exactly which layer of the skin is thinned, or what part of the manipulation has induced that skin thinning, is not known. Each device which is placed on the skin will exert a certain pressure, and it is therefore perhaps not the real thickness of the skin that will be measured at that time.

There are other methods of assessing the long-term effects of steroids. It is possible, for instance, to measure the suppleness of the skin. We did some of this work, and have tried to make some comparison between the early and the late changes. Usually, but not always, there is a correlation between the speed of appearance of the side-effects and the intensity of the late effects — but what seems to be the general rule will not always represent the true happenings.

Question from the audience: Dr August, future restrictions which will be in force for UK practice in an attempt to control the cost of drugs will mean that the introduction of new steroids will be more difficult. Any new steroid will have to supplant an existing steroid already in the drug formulary. It will have to have some major advantage in use or in cost. Do you not think that even the incidental use of these steroids will be questioned?

Dr August: If you fail to be interested in the hospital formulary. It is up to you and your hospital to ask for something if you are convinced of its value. I think it is an appalling impertinence by a general physician to tell us what we may or may not use. We have had this once or twice in hospitals I serve, but in general I think they understand that if you feel you want something you should have it. Just as they would not like to be told what to use, say, on the lung. I think you have to go out and fight for what you want.

Dr P.C. van Voorst (The Netherlands): I found Dr August's presentation interesting because such a personal view may generate a lively discussion. He stated that Locoid® is often prescribed in The Netherlands because it is manufactured there. On the other hand, I suspect that Dr August mentioned those steroids in his presentation because they are manufactured in the UK. Perhaps, however, a supranational approach should be taken, especially with regard to paediatric dermatology which Dr August mentioned. This is an important field in terms of problems with topical steroids, in which preparations both of the Dutch and of the UK firms could be of value.

Could you give any more recently available information about the clinical effectiveness of Preferid® as compared to Dermovate® (clobetasol propionate)? Is anything known about their clinical efficacy?

Dr Murray: Dermovate® is clearly in a class of its own. It is a very potent steroid, more potent than Preferid®. Preferid® is much closer to Diprosone® (or Diproderm®) (betamethasone dipropionate) in potency, and it is certainly more potent than betamethasone-17-valerate in the clinical studies that we have done. That is fortunately quite a clear position.

Question from the audience: Dr Brattsand, I was fascinated by the idea how we could look at steroids and how they worked on the lung and on the skin and then think backwards. You are suggesting that if there was a steroid that could be hydrolyzed or in some way inactivated more quickly, there would be less unwanted effects. What I could not understand is that you did not take into account the depot effect in the epidermis and the fact that the steroid filters through from this depot for another 2 weeks after it has arrived there. Is not the penetration through the epidermis and the fact that the steroid continues for a long while in skin and not in lung presumably one of the major factors in those unwanted effects?

Dr Brattsand: There are two ways by which such effects might be regulated. First, perhaps by using a different contact time that may be required to induce some but not all the effects. That is the case, for instance, in the lung where pulse exposure can be applied, and where some anti-inflammatory effects can be induced without getting a prolonged concentration.

Theoretically, that would be of value in skin if it

was possible to get a very rapid penetration which stopped after a time. However, we are not close to doing that; it is not at this moment a realistic possibility.

The second, and only realistic possibility for differentiation arises because, as we have heard, many important therapeutic effects may happen in the stratum Malpighii in the epidermis. If steroids could be found that are naturally broken down continuously during the absorption process, but still reach sufficient concentration at that layer, and which could further be reduced in the dermis where there is perhaps not the same need for the same high concentration from the therapeutic point of view, then we could have exactly what we need.

However, we do not yet have such steroids. For example, I mentioned that prednicarbate causes atrophy of the skin on sites with a high permeability, which in itself is a condition for even more rapidly biotransformed compounds. This is of course only a theoretical assumption. I have no valid evidence for it, but I think the principle can be used to develop moderately potent steroids that are poorly absorbed or where the small portion that is absorbed on intact skin is biotransformed during the absorption procedure so that there are neither therapeutic effects nor side-effects. This may offer some possibilities for improvement.

Dr Ashworth: With regard to the question about the depot effect and one or two earlier questions about the reason for developing more steroid molecules, in my opinion the vital question is where the steroid will eventually exert its action, rather than whether or not it will be further absorbed systemically. Is this not a question of the transport ability of the vehicle rather than of the intrinsic properties of the steroid molecule itself?

Dr Brattsand: I am not familiar with the pharmaceutical aspects of steroid preparations. I think that the vehicle is important only for the transfer of the steroid through the stratum corneum, after which it does not do much. The further effects of the steroid therefore depend much more upon the inherent properties of blood supply and metabolic turnover at the site of application.

Prof. Havu: We can establish from this session that corticosteroids remain the most potent topical anti-inflammatory agents that we have, and will probably remain so for a long time to come. Constant progress is taking place in research, and we can only guess what we will have in our hands after some years.

I would like to thank all the speakers, discussants and the audience who took part in this part of the symposium. Thank you very much.