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Preclinical evaluation of a new topical corticosteroid methylprednisolone aceponate

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Abstract

Objective: Preclinical characterization of methylprednisolone aceponate.

Results: The local antiinflammatory potency of methylprednisolone aceponate was equal to the very strong glucocorticoid clobetasol 17-propionate but higher than the potency of hydrocortisone 17-butyrate after topical application in 2 animal models of inflammation. Methylprednisolone aceponate is activated enzymatically in the skin. This activation proceeds faster in inflamed tissue. In contrast to clobetasol 17-propionate, methylprednisolone aceponate was devoid of systemic effects after topical application for 3 days. Finally, whereas clobetasol 17-propionate induced marked skin atrophy, methylprednisolone aceponate induced only slight atrophogenic changes after long-term application (up to 43 days) on rat skin, comparable to the effects of hydrocortisone 17-butyrate.

Conclusions: Methylprednisolone aceponate combines high local antiinflammatory potency with very low systemic side effects and only minor local atrophogenic activity. The reason for the dissociation between local antiinflammatory and atrophogenic effects is not known so far. It may be speculated that one of the reasons for the very strong local antiinflammatory activity may reside in the faster enzymatic activation in inflamed tissue. Methylprednisolone aceponate represents a new corticosteroid with which it is possible to improve the dissociation between desired antiinflammatory activity and undesired side effects of topical glucocorticoids.

Key words: Synthetic glucocorticoid; Skin; Inflammation; Esterases; Atrophy

Introduction

Topical glucocorticoids represent the backbone of eczema therapy. Whilst highly potent glucocorticoids for topical use are available, their drawback clearly resides in their potential local and systemic side effects. It has been the aim of pharmacological research to design new compounds combining high antiinflammatory potency and reduced local and systemic side effects.

Glucocorticoids exert their actions through an intracellular receptor which is activated after binding of the steroid. The activated receptor dimerizes, binds to matching DNA-

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sequences and consequently modulates the expression of specific genes. There are no indications that different glucocorticoid receptors are involved in the induction of anti-inflammatory and local or systemic side effects. Therefore, from the pharmacological point of view, the dissociation between desirable and undesirable glucocorticoid actions has to be based on the modulation of the pharmacokinetic characteristics of new compounds [1].

Glucocorticoids for topical use were optimized by chemical modifications for enhanced local activity by different ways. Firstly, their receptor binding affinity, an indicator of their intrinsic activity [2,3] was increased. Secondly, the lipophilicity of glucocorticoids was raised, mainly through esterifications. More lipophilic compounds may penetrate more readily into the skin and may be better retained within the skin [4]. In order to reduce systemic activity, low systemic resorption, rapid systemic inactivation and excretion of glucocorticoids are desired.

Due to the twofold esterification the new glucocorticoid MPA is characterized by a very high lipophilicity. MPA undergoes a distinct metabolism in the skin. Its major metabolite is methylprednisolone 17-propionate, a compound with increased binding to the glucocorticoid receptor. Thus this compound may be expected to possess a higher intrinsic activity [5].

The present paper summarizes our knowledge of the preclinical pharmacology of MPA. Some of the data incorporated have been published previously [6,7]. In addition, we addressed the question whether an inflammatory reaction may influence the rate of esterolytic activation of MPA in the skin.

Material and methods

Croton oil-induced edema

A plastic collar was fixed around the neck of Wistar rats of either sex (160–200 g body weight) to exclude oral uptake of the compounds. Fifty μl of 5% croton oil in ethanol or ethanol alone were topically applied to both ears. In the treatment groups drugs were coapplied with croton oil. Five h after treatment the animals were sacrificed by CO_2 gas and the ears were removed. Edema formation was measured by the increase in wet weight. The IC_{50} values of the antiedematous effects were calculated from the individual values by regression/covariance analysis.

Oxazolone-induced Evans blue extravasation

Female NMRI-mice were sensitized by topical application of 50 μl of 4% oxazolone in ethanol to 4 cm^2 of the shaved left flank. After 13 days the animals were injected intravenously with 0.2 ml of 0.5% Evans blue in water and 20 μl of 4% oxazolone in ethanol were topically applied to 6 cm^2 of the right flank immediately after injection. Three h later the challenged skin was treated with drugs in Neribas® ointment. The animals were sacrificed 24 h after treatment and the challenged skin removed. Evans blue extravasation was measured spectrophotometrically at 623 nm. IC_{50} values for anti-oedematous effects were calculated from the individual absorbancy values by regression/covariance analysis.

Esterolytic activity

Female Wistar rats were sacrificed by CO_2 gas 16 h after topical application of 20 μl of 5% croton oil in ethanol or ethanol alone to both ears. The ears were removed and homogenized in 6 ml 0.1 mol/l phosphate buffer, pH 7.4 under constant cooling. After centrifugation at $20000 \times g$ for 20 min the supernatants were frozen in liquid nitrogen and stored at -20°C until use.

The reaction was started by addition of 20 μl of 5 mmol/l compound in ethanol to 1 ml

supernatant. After incubation at 37°C the samples were placed on ice and extracted 3 times with 3 ml chloroform. Twenty μl of an internal standard was added to the reaction mixture immediately before the extraction. The organic phases were pooled, evaporated under nitrogen and dissolved in 100 μl ethanol. Substrates and metabolites were separated by HPLC on a reversed phase column with a gradient of acetonitrile and water containing 0.015% trifluoroacetic acid. The relative amount of each compound was calculated from the peak areas. Data were not corrected for traces of metabolites in the substrate used.

Systemic effects after short-term application

Four to five cm^2 of the dorsal skin of female Wistar rats, body weight 140–160 g, was shaved. The animals were treated once daily for 3 days with 100 μl of test compound at a concentration of 0.1% in Neribas® ointment or with the base alone. The treated skin was covered by a plastic sheet. The animals were sacrificed one day after the last treatment by CO_2 gas. The systemic activity of the compound was measured by the decrease in wet weight of thymus and spleen.

Local and systemic effects after long-term application

A polypropylene collar was fixed around the neck of female Wistar rats (120–140 g) prior to use to exclude oral uptake of the compounds. Ten μl of test compound at a concentration of 0.2% was applied once daily to 1 cm^2 of the shaved dorsal skin. The body weight was determined every 2–3 days. The animals were sacrificed by CO_2 gas 10, 20, 30 and 43 days after treatment. The systemic activity of the compound was measured by the decrease in wet weight of thymus, spleen and adrenal glands. The reduction of skin thickness and breaking strength was determined as a measure for the local atrophogenic activity. The difference between treatment groups was evaluated for statistical significance at the 95% confidence level.

Table 1
Local antiinflammatory effects of topically applied glucocorticoids

Glucocorticoid	IC_{50} [%] [a]	
	Coroton oil-induced edema	Oxazolone-induced Evan blue extravasation
CBP	0.0018	0.05
MPA	0.0015	0.04
HCB	0.005	0.12

[a] Concentration of glucocorticoid in % (w/v) in the formulation necessary to inhibit the inflammatory reaction by 50%.

Results

The local antiinflammatory activity of methylprednisolone aceponate (MPA) was

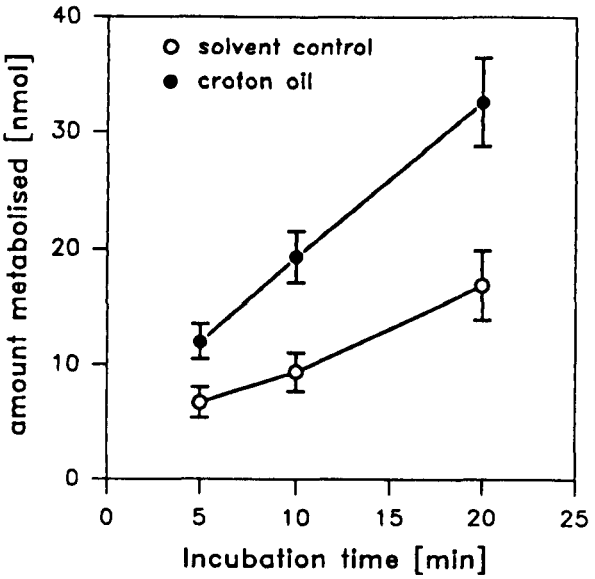

Fig. 1. Metabolism of methylprednisolone aceponate in homogenates of solvent and croton oil-treated rat ears. Results are given as mean ± standard deviation from determinations with ears of 6 animals in each group. Data were not corrected for traces of metabolites in the substrate used.

Table 2
Hydrolysis of hydrocortisone 21-acetate in homogenates of control and inflamed ears

Incubation time (min)	nmol substrate hydrolysed [a]	
	solvent control	croton oil
5	2.0±0.6 [b]	3.8±0.9
15	8.9±2.6	16.0±5.5
60	21.0±2.7	34.7±7.9

[a] Results are given as mean±standard deviation from determinations with ears of 6 animals in each group.
[b] Ears of only 4 animals were included in this incubation.

compared to the very strong glucocorticoid clobetasol 17-propionate (CBP) and the medium potency hydrocortisone 17-butyrate (HCB). The results are given in Table 1. In both test systems, the croton oil-induced edema and the oxazolone-induced Evans blue extravasation, MPA was equipotent to CBP and more potent than HCB. The difference between MPA and HCB reached statistical significance, however, only in the rat ear test.

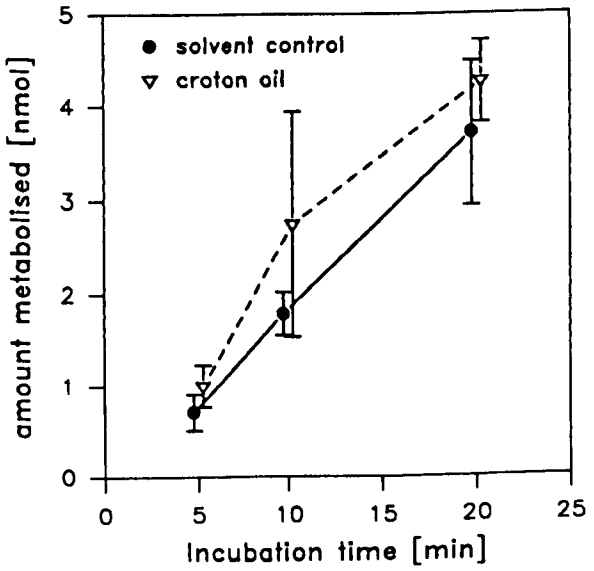

Fig. 2. Conversion of methylprednisolone 17-propionate in homogenates of solvent and croton oil-treated rat ears. Results are given as mean±standard deviation from determinations with ears of 6 animals in each group.

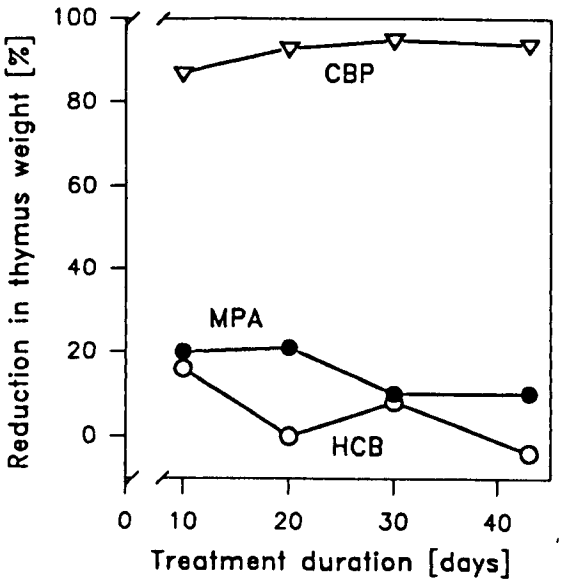

Fig. 3. Reduction in thymus weights in rats after long-term topical treatment with HCB, MPA and CBP. The animals were treated daily with 10 μl of a 0.2% (w/v) solution of the indicated glucocorticoids spread over 1 cm^2 of back skin.

When MPA was incubated in homogenates from rat ears for up to 20 min, the 21-acetate group was rapidly hydrolysed resulting in the formation of methylprednisolone 17-propionate as the major metabolite. In addition, such incubations resulted in the formation of minor amounts of methylprednisolone 21-propionate and methylprednisolone. Both latter compounds, however, amounted to less than 5% of the major metabolite methylprednisolone 17-propionate. In homogenates of rat ears treated with croton oil, the hydrolysis of MPA proceeded at about twice the rate of the hydrolysis observed in homogenates of solvent treated ears (Fig. 1).

Similarily, incubation of hydrocortisone 21-acetate in rat ear homogenates resulted in a rapid formation of hydrocortisone by hydrolysis of the 21-acetate. As observed for MPA the esterolytic hydrolysis of hydrocortisone 21-acetate was increased in homogenates of inflamed ears (Table 2). These

findings indicate an increase in the esterolytic capacity in the inflamed skin tissues.

In contrast, the rate of formation of methylprednisolone 21-propionate and methylprednisolone from methylprednisolone 17-propionate was very low and did not differ between incubations with homogenates of control and inflamed ears (Fig. 2).

When MPA and HCB were applied topically for 3 days at a concentration of 0.1% in Neribas® ointment to the skin of rats, the thymus and spleen weights were not significantly reduced, indicating that these glucocorticoids exerted no systemic activity after short-term application, whereas CBP induced a reduction of thymus and spleen weights by 74 and 46%, respectively (data not shown).

The systemic and local side effects of MPA, HCB and CBP were also studied after long-term application of 0.2% solutions in a mixture of ethanol and isopropylmyristate (95:5). After 43 days, MPA or HCB had no significant influence on the growth of the animals during this period, whereas application of CBP resulted in a complete prevention in weight gain and even led to a reduction in body weight (data not shown). Skin thickness was reduced to a similar degree (33% to 48%) after 43 daily applications of MPA, HCB and CBP (data not shown). In addition, the influence of MPA, HCB and CBP on thymus weight (systemic side effects) and on skin breaking strength (local side effects) was studied at different time points. The time course of the effects of glucocorticoids on thymus weight (Fig. 3) indicates that this parameter of systemic activity of glucocorticoids is already maximally affected after 10 days. The skin breaking strength, however, decreased with glucocorticoid treatment progressively over the whole observation period of 7 weeks (Fig. 4) indicating a slow effect of glucocorticoids on this parameter.

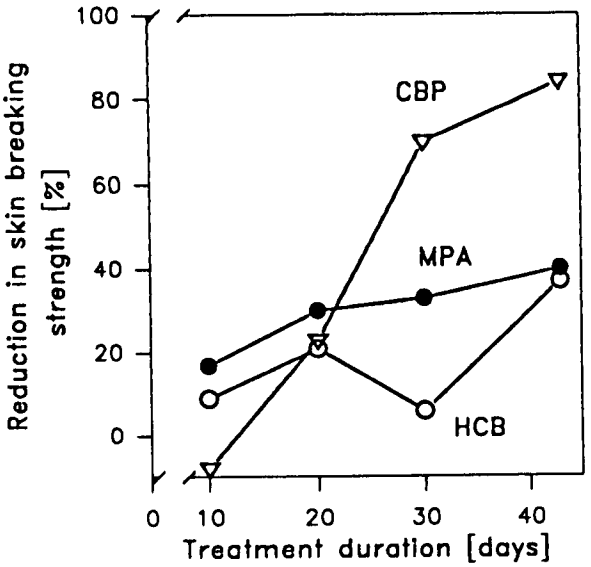

Fig. 4. Reduction in skin breaking strength after long-term topical application of HCB, MPA and CBP. For experimental conditions please refer to text or legend of Fig. 3.

Discussion

The local antiinflammatory activity of MPA was investigated in two different animal models of inflammation, the croton oil-induced edema in rat ears [8] and the oxazolone-induced delayed type hypersensitivity reaction [9] in mice. In both assays MPA was equipotent to the very strong glucocorticoid CBP. In contrast, MPA was significantly more potent than HCB in the rat ear test and numerically more potent than HCB in the oxazolone test. The relative potencies of the three glucocorticoids were similar in both animal models of skin inflammation involving different species, application sites and vehicles, indicating that the ranking in potency for the tested glucocorticoids was not due to particularily favourable conditions in a unique assay.

It has been reported that MPA undergoes an extensive esterolytic metabolism in the skin [5]. The rate of this esterolytic metabolism of MPA was found to be dou-

bled in homogenates of inflamed rat ears when compared to homogenates of solvent-treated controls. Similar results were obtained upon incubation of hydrocortisone 21-acetate with homogenates of inflamed rat ears, indicating that our observation of an increased esterolytic hydrolysis may apply for other glucocorticoid 21-acetates as well. In contrast, the primary metabolite of MPA, the glucocorticoid 17-ester methylprednisolone 17-propionate, was hydrolysed at a much lower rate and there was no difference between the rates of apparent metabolism in control and inflamed ears. This observation is in accordance with the view that glucocorticoid 17-esters are not directly cleaved by esterases but only after a chemical acyl-migration to the corresponding glucocorticoid 21-ester [5,10].

An increase in esterolytic activity in inflamed tissues is not unexpected. There are, however, a number of possible sources for the additional esterolytic activity. Firstly, this enzymatic activity may stem from serum components [5] which may exudate into the skin in edematous reactions. Secondly, histochemical results point to leukocytes [11], which infiltrate the skin in inflammatory reactions [12,13], as a potential source of esterolytic activity. Thirdly, increased esterolytic activity has been reported in dermal fibroblasts participating in tissue remodelling after experimental injury [14]. Thus, the origin of the additional glucocorticoid ester hydrolysing activity remains to be clarified. To the best of our knowledge, the present paper is the first to report an increased esterolytic activation of glucocorticoids in inflamed tissue.

Whether changes in glucocorticoid metabolism in human skin occurs during inflammation is presently unknown. Since the increase in the esterolytic cleavage of tosyl-arginine-methyl ester is a sensitive parameter for allergic reactions in nasal secretions [15], however, one may speculate that inflammatory reactions may modulate the esterolytic metabolism in human tissues as well.

The influence of the faster hydrolysis in inflamed tissue on the therapeutic quality of the compound is difficult to predict, because the metabolism of compounds in the skin may also influence their penetration through the skin [16]. It may, however, be speculated that a faster esterolytic activation in the inflamed skin could favourably modulate the antiinflammatory activity of MPA, because the primary metabolite of MPA, methylprednisolone 17-propionate, has a higher affinity to the glucocorticoid receptor than the parent glucocorticoid diester [5].

Systemic glucocorticoid activity after topical application of MPA was not detected either after application for 3 days or after application for 43 days. This was also true for HCB, a compound with lower local antiinflammatory activity than MPA. In contrast, CBP, a glucocorticoid with the same local antiinflammatory potency as MPA in our test systems, induced marked systemic effects after topical application at the same concentrations as MPA. This indicates a very favourable dissociation of the local antiinflammatory from the systemic activity of MPA. The strong local antiinflammatory activity of MPA may be due to a combination of its very high lipophilicity [6], which may facilitate its penetration into the skin, and its esterolytic activation, which may proceed faster in inflamed skin and can increase the intrinsic activity of the glucocorticoid. The low systemic activity may be explained by the rapid elimination of the primary metabolite methylprednisolone 17-propionate from the circulation after glucuronidation (cf. Täuber, this issue).

The reduction in skin breaking strength proceeded very slowly in comparison to the rapid reduction in thymus weight. This may be due to the low turnover rate of collagen in rat skin, which has been estimated to be in the range of 1000 days [17].

Compared to CBP, both MPA and HCB induced less atrophogenic effects after long-term application to rat skin. This points to a favourable dissociation of local antiinflammatory and atrophogenic activity of MPA. The reason for the apparent selectivity of MPA for the local antiinflammatory activity rather than the induction skin atrophy is at present unknown.

In conclusion, the preclinical data characterize the modern glucocorticoid diester MPA as a glucocorticoid with a marked dissociation between local antiinflammatory activity and activity to cause side effects.

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