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Original Article

Superior nuclear receptor selectivity and therapeutic index of methylprednisolone aceponate versus mometasone furoate

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Abstract: Although introduced more than 50 years ago, topical glucocorticoids are still the first line therapy for many inflammatory skin disorders such as atopic eczema, contact dermatitis and many others. Recently, significant improvements have been made to optimize the ratio of desired to unwanted effects. While with early compounds such as triamcinolone, topical side effects such as skin atrophy and telangiectasias can be observed rather frequently, newer drugs such as methylprednisolone aceponate or mometasone furoate have a significantly improved therapeutic index. The present study compared these two modern topical glucocorticoids, which possess the highest therapeutic index currently found, in terms of nuclear receptor selectivity *in vitro* and induction of the most important local side effects (skin atrophy and telangiectasias) in a relevant rodent model *in vivo*. We demonstrate that methylprednisolone aceponate displays higher specificity in nuclear receptor binding compared with mometasone furoate. Methylprednisolone aceponate was also markedly superior in terms of minimizing induction of skin atrophy or telangiectasias when compared with mometasone furoate. Based on these observations, methylprednisolone aceponate is expected to have a greater therapeutic index as compared with mometasone furoate, at least in the test systems used here. The degree to which this observation may translate into a clinical setting requires confirmation.

Key words: glucocorticosteroid – skin atrophy – skin inflammation – telangiectasia – therapeutic index

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Introduction

Glucocorticoids (GCs) are the most widely used class of anti-inflammatory drugs. In the early 1950s, hydrocortisone was observed to be an effective topical therapy of inflammatory skin disorders. This initial success spurred the evolution of compounds with higher potency. Triamcinolone acetonide was one of the first halogenated corticosteroids that led to the development of the superpotent GCs (1,2).

Abbreviations: AR, androgen receptor; GC, glucocorticoid; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; NR, nuclear receptor; HPA, hypothalamic-pituitary-adrenal; MF, mometasone furoate; MPA, methylprednisolone aceponate; TIX, therapeutic index; MPP, 6α-methylprednisolone-17-propionate; DEX, dexamethasone; CCS, charcoal-stripped calf serum; CF, competition factor; nGSR, non-GC-selective receptor.

Although these superpotent GCs were highly effective for certain indications, localizations and types of disease, they showed harmful local and systemic side effects when used over prolonged periods or when improperly applied. In terms of local side effects, skin atrophy is one of the most important side effects in topical GC therapy (3–6). Skin atrophy compromises the skin's function to maintain a permeability barrier between the organism and the external environment (7). GC-induced skin atrophy is characterized by increased skin tearing, thinning and telangiectasia (8).

Glucocorticoid atrophogenic potential is commonly assessed in a preclinical, *in vivo* rat model (2,8,9). Current studies prefer hairless animals such as *hr/hr* rats because of the unreliability of haired skin responses (10). Skin thickness is one parameter for skin atrophy, determined over time in the living animal, using a specifically designed gauge to measure skin fold thickness by applying a defined pressure to ensure reproducibility (11). Skin breaking

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strength measurement of GC-treated skin is used as an additional parameter for skin atrophy (10).

In comparison with human skin, the hairless rat skin is more sensitive to atrophy following topical GC application. Anatomical differences between the rodent and human skin, such as increased dermal layers in human skin, may influence pharmacokinetics (2,12). Using this model has helped gauge the evolution of GCs.

Recently, the therapeutic index (TIX) has been established as a standard for topical GCs by measuring both the desired anti-inflammatory effects and the unwanted side effects (13). The concept of the benefit to risk ratio evaluation with topical GCs is based on *in vitro* and *in vivo* data with regards to efficacy and safety (14–16). The TIX value is indicated as a ratio of desired to adverse effects, thus a ratio approximating 1 signifies an equal relation of desired and adverse effects, whereas a ratio of 2–3 indicates a GC with increased benefit to risk ratio. With increasing knowledge of the mechanism of action of GCs and pharmacokinetics of the skin, GC development turned towards compounds with a better TIX, leading to compounds such as prednicarbate, methylprednisolone aceponate (MPA) and mometasone furoate (MF) with the highest TIX of 2.0; here we focus on the latter two compounds.

Methylprednisolone aceponate (Fig. 1a) and MF (Fig. 1b) are both applied once daily as 0.1% topical cream, ointment, or lotion formulations for the treatment of patients with inflammatory GC-responsive dermatoses (17,18). Compared with previous generations of GCs, MPA and MF exhibit marked improvement with regards to lipophilicity, local and systemic side effect profile and TIX score. Lipophilicity has increased as a function of esterification, onefold in MF and twofold in MPA (19). Both compounds present an increased TIX value as a function of decreased side effect profiles coupled with potent anti-inflammatory activity. Major systemic side effects, such as hypothalamic-pituitary-adrenal (HPA) axis suppression and decreased serum cortisol levels, have also been minimized (19). However, the fundamental structural differences between MPA and MF offer the opportunity for further differentiation.

Highly lipophilic agents, such as MPA and MF, generally do not attain high serum concentrations, thus reducing the potential for systemic side effects (20). MPA shows a marked improvement in lipophilicity because of its twofold esterification compared with the onefold esterification in MF. Consequently, MPA shows increased penetration into the skin and exhibits high local anti-inflammatory activity. Once in the skin, esterases hydrolyse MPA forming 6α-methylprednisolone-17-propionate (MPP), a more potent corticoid than MPA. Bioactivation of MPA is accelerated in inflamed skin relative to normal skin because of higher levels of esterases found in inflamed skin, leading to a further increase in active MPP concentration at the site

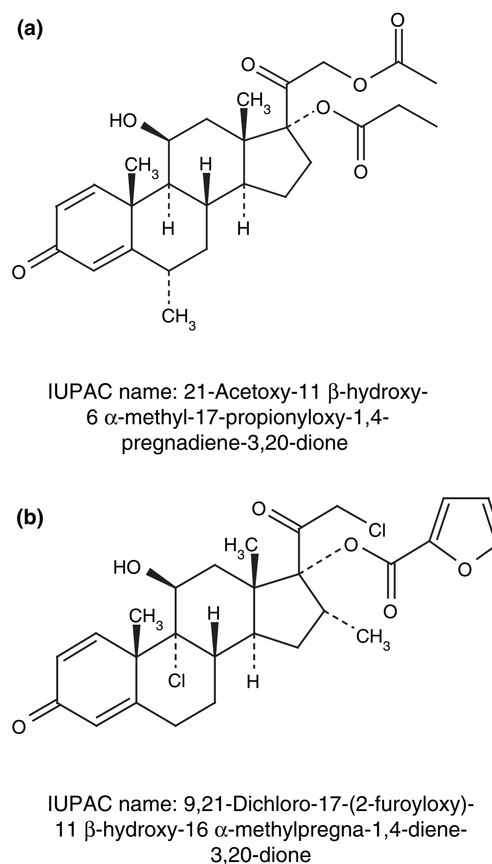

Figure 1. Chemical structures of (a) methylprednisolone aceponate and (b) mometasone furoate.

of inflammation (20). In contrast, MF's potent anti-inflammatory activity can be partly attributed to the chlorine moiety present at the C-21 position, which inhibits esterase activity leading to high compound retention at the application site (17,21).

As the MPA metabolite (MPP) passes through the skin and enters the blood, glucuronic acid rapidly inactivates MPP by conjugation followed by excretion mainly through urine, which explains the low systemic activity of MPA (22). Although MF does not cause marked systemic side effects, only 0.00076% of the total administered dose is excreted in urine as the metabolite, 6β-hydroxy mometasone (23). Furthermore, Sahasranaman et al. (24) reported that orally inhaled and intravenously administered MF might produce higher systemic exposure than originally anticipated because of the presence of active metabolites generated from distinct extrahepatic metabolism, which may be partly responsible for MF's systemic side effects (24).

Indeed, MPA and MF are appreciably better topical GCs than many predecessors; however, MPA seems to display an even better local side effect profile than MF, one of the most important parameters being skin atrophy. Kecskes et al. (19) reported that MPA causes significantly less

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atrophy and significantly less severe telangiectasias than MF. This article provides additional data supporting this claim by examining skin atrophy, breaking strength and telangiectasias as a result of MPA and MF administration at equipotent and equiefficacious concentrations using a rat model of GC-induced skin atrophy. We show here that MPA displays less non-specific receptor binding and transactivation as an *in vitro* measure of side effect potential in addition to lower atrophogenic potential and less severe telangiectasias formation *in vivo*.

Materials and methods

Receptor-binding assays

Cytosol preparations of Sf9 cells, infected with recombinant baculovirus coding for the human glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), or androgen receptor (AR), were used. After centrifugation (15 min, 600 g), Sf9 pellets were resuspended in 1/20 volume of 20 mM Tris–HCl, pH 7.4/0.5 mM EDTA/2 mM dithiothreitol (DTT)/20% glycerol/400 mM KCl/20 mM sodium molybdate/0.3 μM aprotinin/1 μM pepstatin/10 μM leupeptin and shock frozen in liquid nitrogen. After three freeze/thaw cycles, the homogenate was centrifuged for 1 h at 100 000 g. Protein concentration of the resulting supernatant was between 10 and 15 mg/ml. Aliquots were stored at −40°C.

For the binding assays of GR, MR, PR and AR, [^{3}H]dexamethasone (DEX) (≈20 nM), [^{3}H]aldosterone, [^{3}H]progesterone, or [^{3}H]methyltrienolone, respectively, and Sf9 cytosol (100–500 μg protein), test compound and binding buffer (10 mM Tris–HCl, pH 7.4/1.5 mM EDTA/10% glycerol) were mixed in a total volume of 50 μl and incubated for 1 h at room temperature. Specific binding was defined as the difference between binding of [^{3}H] DEX, [^{3}H]aldosterone, [^{3}H]progesterone and [^{3}H]methyltrienolone in the absence and presence of increasing concentrations (3×10^{-10}, 1×10^{-9}, 3×10^{-9}, 1×10^{-8}, 3×10^{-8}, 1×10^{-7}, 3×10^{-7} and 1×10^{-6} M) of unlabelled DEX, aldosterone, progesterone, and metribolone. After incubation, 50 μl of cold dextran-coated charcoal suspension was added for 5 min, and the mixtures transferred to microtiter filtration plates. The mixtures were filtered into Picoplates (Canberra Packard, Dreieich, Germany) and mixed with 200 μl Microszint (Canberra Packard). The bound radioactivity was determined with a Packard Top Count plate reader. The concentration of test compound giving 50% inhibition of specific binding (IC_{50}) was determined from Hill analysis of the binding curves (10).

Transactivation activity assays

Oestrogenic activity was measured in MCF7 cells expressing endogenous estrogen receptor (ER)α stably transfected with oestrogen-sensitive promoter reporter gene construct (pvit-tk-luciferase); oestradiol and fulvestrant were used as agonist and antagonist reference compounds respectively. Progestenic activity was measured in SK-N-MC cells stably transfected with human PR-B and a reporter gene (MMTV-luciferase); Promegestone and Mifepristone were used as agonist and antagonist reference compounds respectively. Androgenic activity was measured in CV-1 cells stably transfected with rat AR and reporter gene (MMTV-luciferase); metribolone and cyproterone acetate were used as agonist and antagonist reference compounds respectively. Mineralocorticoid activity was measured in COS cells transiently transfected with human MR and reporter gene (MMTV-luciferase); Aldosterone and ZK 91587 (7α-Methoxycarbonyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone) were used as agonist and antagonist reference compounds respectively. All activity was measured as light units emitted by luciferase.

Briefly, cells were grown in DMEM supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 4 mM L-glutamine and 10% fetal calf serum (FCS) (Invitrogen, Carlsbad, CA, USA), SK-N-MC cell media system included 1 mM sodium pyruvate and non-essential amino acids. MCF-7 cells were preincubated for 24 h with media plus 5% charcoal-stripped calf serum (CCS) and 1×10^{-9} M Fulvestrant (antagonist) to reduce high signals; the media was subsequently replaced by media plus 3% CCS for 24 h before beginning experiment. Prior to experiments for the remaining cell lines, cells were plated from 1×10^4 to 2×10^4 cells per well in 96-well dish and incubated in stated media plus 3% CCS for 24 h; SK-N-MC cell test system media included 25 mM HEPES and incubation time was carried out to 48 h. Agonistic activity was tested by adding increasing concentrations of MPA or MF (1×10^{-12} to 1×10^{-6} M); as a positive control, the appropriate agonist was added at increasing concentrations (1×10^{-12} to 1×10^{-6} M), 0.1% dimethyl sulfoxide (DMSO) was used as a negative control; experiments were carried out to the 24 h time point. Antagonistic activity was tested by treating cells with a single concentration of appropriate agonist to system (1×10^{-10} M oestradiol, promegestone and aldosterone; 5×10^{-10} M metribolone) and adding the increasing concentrations of MPA or MF as before. As a positive control for inhibition, cells were treated with increasing concentrations of appropriate antagonist (1×10^{-12} to 1×10^{-6} M); 0.2% DMSO was used as a negative control, experiments were carried out to the 24 h time point.

Luciferase activity was measured by lysing cells with 20 μl Cell Culture Lysis Reagent (Luciferase Assay System; Promega, St Luis Obispo, CA, USA). The activity of the luciferase reporter gene product as determined in cell lysates is described by the manufacturer (Promega).

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Animal models

Animals
Wistar and juvenile, hairless rats (*hr/hr*, 80–100 g) were obtained from Charles River, Italy. Rats were housed and experimental procedures were performed according to institutional guidelines; animals had access to food and water *ad libitum*.

Anti-inflammatory activity
Tetradecanoylphorbol acetate is the active component of croton oil commonly used as an irritant in skin inflammation models to test compounds for their anti-inflammatory activity after oral or topical administration. Female Wistar rats ($n = 10$/group) were topically treated with croton oil (Fluka, Schellendorf, Germany) solution (6.5% v/v) in a total volume of 0.04 ml EtOH. Compounds or vehicle were dissolved in the croton oil solution and topically co-applied to both ears. After 24 h, animals were killed and the weight of 10-mm ear punch biopsies as an overall read-out of inflammation (oedema) was determined; experiments were repeated for three (3) times.

Local side effects following prolonged compound application
Two concentrations of the active compounds were chosen: (i) an equipotent concentration defined as $3 \times ED_{50}$, the threefold concentration at which there is a 50% inhibition of oedema formation as determined in the croton oil rat model (0.035% for MPA, 0.0027% for MF) and (ii) an equiefficacious concentration which results in approximately 80% inhibition of oedema formation in the croton oil rat model (0.1% for MPA, 0.01% for MF). Ten to 13 rats were randomly allocated to the different treatment groups. Three days prior to compound application, rats were provided with flexible collars to prevent oral ingestion of the topically applied compound by licking. A treatment area of 3×3 cm^2 on the proximal back of the animal was tattooed with ink using an 18-gage needle. Compounds were dissolved in isopropylmyristate-containing ethanol (5:95, v/v) and applied once daily for 19 days between 7 and 10 AM on the marked treatment area at 75 μl total volume. Three parameters were measured as local side effect readouts: (i) skin fold thickness, (ii) skin tensile strength and (iii) telangiectasias.

Skin fold thickness was measured immediately prior to the first compound application on day 1; animals were weighed and skin fold thickness was determined to establish baseline values. These measurements were repeated on days 5, 8, 12, 15 and 19. Measurements were made with a pressure-controlled, automated dial thickness gauge (Schering AG, Berlin, Germany). Caliper contact pressure was fixed to 100 p. Mean values of skin fold thickness were derived by averaging two adjacent treated skin areas (10).

Skin tensile strength was determined as previously detailed (25). Briefly, at the end of the experiment, on day 20, animals were killed using CO_2 gas, and a 5×5 cm^2 area of the back containing the treated area was removed. The skin patch was placed on filter paper and two double-T-shaped skin pieces were punched out (5-cm long and 0.4-cm wide at the narrowest point) transverse to the longitudinal axis of the body. The skin strips were covered with moistened filter paper to avoid drying and were fixed with their wider ends into an in-house developed tensile tester. The force necessary to tear the skin strip at a constant stretch rate of 200 mm/min was determined with a pressure sensor and was expressed as the skin-breaking strength (N) (10).

Telangiectasias were measured on day 20, after animals were killed. The treated skin areas were photographed using a digital camera. Length of telangiectasias were measured (in pixel) by a blinded investigator not involved in the performance of the *in vivo* experiments. Experiments to measure cutaneous side effects following topical GC application were repeated twice with at least 10 animals per group.

Statistics

All data satisfied Bartlett's test to exclude different variances of the study populations; data were analysed by ANOVA with Tukey's multiple comparison post test using the SISAM program developed by Schering's Department for Biometrics (10) which is based on SAS for Windows 6.12 (SAS Institute, Cary, NC, USA). Outliers were determined according to Dixon's r_{10} outlier test (26) using the formula:

$$r_{10} = d_{\max}/r$$

where r_{10} is the critical value that depends on sample size and a-level (e.g. $r = 0.412$ for $a = 0.90$), r is the range and $d_{\max}$ is the maximal difference between the two lowest or two highest values. Experiments were repeated twice; one representative example is depicted. Figures show mean values ± SD if not mentioned otherwise.

Results

In vitro receptor binding profile

Both compounds' non-specific affinity (IC_{50}) for three nuclear receptors – human PR, rat AR and human MR – was measured and evaluated as a ratio against the specific reference compound for each receptor. This ratio is referred to as a competition factor; a larger value indicates a relatively higher IC_{50} of the test compound relative to the reference compound, and thus a weaker non-specific receptor affinity. MF presented competition factor values of 1.9, 48 and 1.6 for PR, AR and MR respectively. MPA displayed a competition factor value of 68 for PR and did not bind AR

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Table 1. Competition factor values of MPA and MF

Test compound	hPR (Progesterone)	hAR (Metribolone)	hMR (Aldosterone)
Prednisolone	n.b.	n.b.	1.5
MPA	68	n.b.	n.b.[1]
MF	1.9	48	1.6

MF, mometasone furoate; MPA, methylprednisolone aceponate; PR, progesterone receptor; AR, androgen receptor; MR, mineralocorticoid receptor; n.b., no binding.
Values represent competition factor – established as the ratio of receptor affinity (IC_{50}) of the test and the reference compound, given in parentheses. Per definition, competition factor is 1.0 for standard compounds.
[1]Due to differences in sensitivity of the cell-free binding assays and the cellular transactivation assays, a low potent activity in transactivation was found although no binding was observed.

or MR to any measurable extent (Table 1). These values indicate that MPA displays a lower non-specific binding profile than MF does.

Transactivation assays

To test the effect of receptor binding, a receptor-dependent transactivation activity assay was performed for MPA and MF, directed towards human PR, MR and rat AR; affinities were measured via EC_{50} values with a greater value meaning lower non-GC selective receptor (nGSR) potency. MPA displayed a larger EC_{50} value for agonism via hPR (0.024 ± 0.005 nM) and hMR (0.026 ± 0.005 nM) relative to MF, 0.0067 ± 0.0008 nM (hPR) and 0.34 ± 0.2 nM (hMR). Both MPA and MF demonstrated an agonism EC_{50} value in excess of 1000 nM for rAR, and an antagonism IC_{50} value in excess of 1000 nM for hPR. MPA exhibited a larger IC_{50} value for antagonism via rAR (>1000 nM) and hMR (>1000 nM) relative to MF, 755 ± 139 nM (rAR) and 0.61 ± 0.2 nM (hMR) (Table 2). These data suggest that the level of nGSR transactivation activity is lower in MPA- versus MF-treated samples relative to specific reference compounds.

Anti-inflammatory activity

To establish efficacy concentrations for MF and MPA, ear weight was measured as an indication of oedema inhibition in response to topical compound application in the croton oil-induced dermatitis model in the rat. An ED_{50} concentration of 0.01% was established for MPA and 0.001% for MF ($P < 0.05$, Fig. 2a). Therefore, we concluded that MF shows tenfold greater potency than MPA in this specific rat model, whereas the two compounds are equipotent in man at the same 0.1% formulation (19).

Table 2. Progesterone, androgen, and mineralocorticoid receptor-dependent transactivation activity (agonistic and antagonistic) of methylprednisolone aceponate and mometasone furoate

	Progesterone receptor				Androgen receptor				Mineralocorticoid receptor			
	Agonism		Antagonism		Agonism		Antagonism		Agonism		Antagonism	
	EC_{50} (nM)	Efficay (%)	IC_{50} (nM)	Efficay (%)	EC_{50} (nM)	Efficay (%)	IC_{50} (nM)	Efficay (%)	EC_{50} (nM)	Efficay (%)	IC_{50} (nM)	Efficay (%)
Reference	Promegestone (R5020)		Mifepristone (RU 486)		Metribolone		Cyproterone acetate		Aldosterone		ZK 91587[1]	
	0.024 ± 0.005 *n* = 5	100	0.039 ± 0.015	100	0.28 ± 0.05 *n* = 5	100	21 ± 5.5	100	0.026 ± 0.005 *n* = 5	100	7.7 ± 0.8	100
MPA	13.0 ± – *n* = 4	68 ± 1.0	125 ± 17.3	28.5 ± 3.3	11.3 ± 1.3 *n* = 4	14.5 ± 1.0	>1000	8.7 ± 9.6	6.2[2] ± 1.1 *n* = 4	97.8 ± 7.6	>1000	na
MF	0.028 ± 0.017 *n* = 6	100.7 ± 3.8	>1000	na	0.91 ± 0.2 *n* = 4	28.1 ± 8.8	412.5 ± 5.0	47.9 ± 6.7	0.64 ± 0.3 *n* = 5	58.0 ± 10.2	3.4 ± 2.8	40.7 ± 10.7

EC_{50} values for agonistic and IC_{50} values for antagonistic activity are listed as mean values ± SD. Efficacies are shown as % of maximum effect of the respective reference. EC_{50} or IC_{50} values >1000 nM indicate no activity in the respective transactivation assay.
[1]ZK91587 is 7-α-Methoxycarbonyl-15β,16β-methylene-3-oxo-17α-pregn-4-ene-21,17-carbolactone.
[2]Due to differences in sensitivity of the cell-free binding assays and the cellular transactivation assays, a low potent activity in transactivation was found although no binding was observed.

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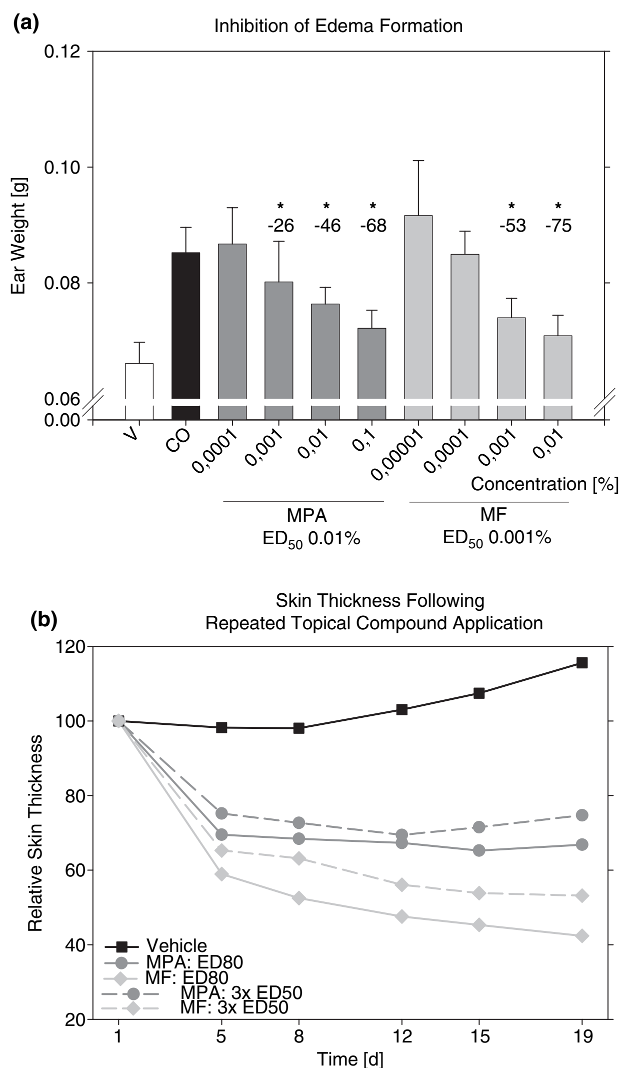

Figure 2. (a) Methylprednisolone aceponate (MPA) and mometasone furoate (MF) show similar efficacy in inhibition of oedema formation in the croton oil-induced dermatitis model in rats. Results show mean values ± SD from one representative out of three independent experiments. *P < 0.05 versus croton oil control (■), values in percentage show inhibition compared with croton oil positive control. Comparing maximum inhibitory effects on oedema formation of MPA (0.1 %) and MF (0.01 %), no significant differences between MPA and MF were found. (b) MPA exhibits lower levels of skin thinning relative to MF; time course of skin atrophy in MPA- (•) and MF- (◇) treated skin of animals that have been topically treated for the indicated time at equipotent (dashed lines, $3 \times ED_{50}$) or equiefficaceous (straight lines, approximately 80% inhibition of oedema formation in croton oil rat model) concentrations of MPA and MF. Data are normalized (skin thickness day 1 = 100%).

Local side-effect profile

Skin thickness is utilized as a parameter for measuring local side effects of topical compound application. Skin thickness in the area of compound application – at equipotent and equiefficacious concentrations – was measured over a 19-day period of daily compound application on days 1, 5, 8, 12, 15 and 19. The greatest change in skin thickness was observed between days 1 and 5. From this point forward, both concentrations of MPA plateaued at about 70% skin thickness relative to that observed on day 1; however, both concentrations of MF displayed a constant decrease in skin thickness with each subsequent measurement relative to day 1 with a final skin thickness of <60% in both concentrations. The vehicle control exhibited a gradual increase in skin thickness which can be attributed to ageing of juvenile rats and natural skin thickening (Fig. 2b). MPA clearly demonstrated a lower degree of skin thinning relative to MF.

Endpoint (day 19) skin thickness examination revealed significantly less skin thinning in MPA- than MF-treated skin relative to vehicle control. At equipotent ($3 \times ED_{50}$) concentrations, MPA resulted in a 34% decrease in skin thickness relative to vehicle control, while MF resulted in a 51% decrease in skin thickness. At equiefficacious (ED_{80}) concentrations, the results were even more disparate; MPA exhibited 39% decrease in skin thickness relative to vehicle control, while MF exhibited 60% decrease in skin thickness (Fig. 3a).

As an additional parameter to gage the local side-effect profile, skin-breaking strength was measured on day 20. These data revealed greater skin tensile strength retention in MPA- versus MF-treated skin relative to vehicle control. At $3 \times ED_{50}$ values, MPA-treated skin showed a 17% decrease in tensile strength while MF-treated skin displayed a 52% decrease in tensile strength. At equiefficacious concentrations, MPA-treated skin showed 22% decrease in skin tensile strength and MF showed a 58% decrease (Fig. 3b).

Telangiectasias were measured as described earlier; the pixilated measurement was then translated to a graph comparing MPA- with MF-treated skin (Fig. 4). MPA resulted in 0.25 relative telangiectasias length compared with 0.75 with MF.

Results indicate that MPA-treated skin better retains thickness and tensile strength, and also displays a lower degree of telangiectasias formation compared with the equiefficacious concentration of MF. We conclude that MPA results in a lower side-effect profile than MF in rodent models used in this study.

Discussion

Methylprednisolone aceponate displays higher specificity in NR binding compared with MF and also presents less GR-mediated topical side effects in a rodent model of skin atrophy at equiefficacious and equipotent concentrations.

Historically, GC anti-inflammatory effects have been accompanied by unwanted side effects – a number of which are predominantly caused by a GR-DNA binding-dependent transactivation mechanism (27,28). In addition

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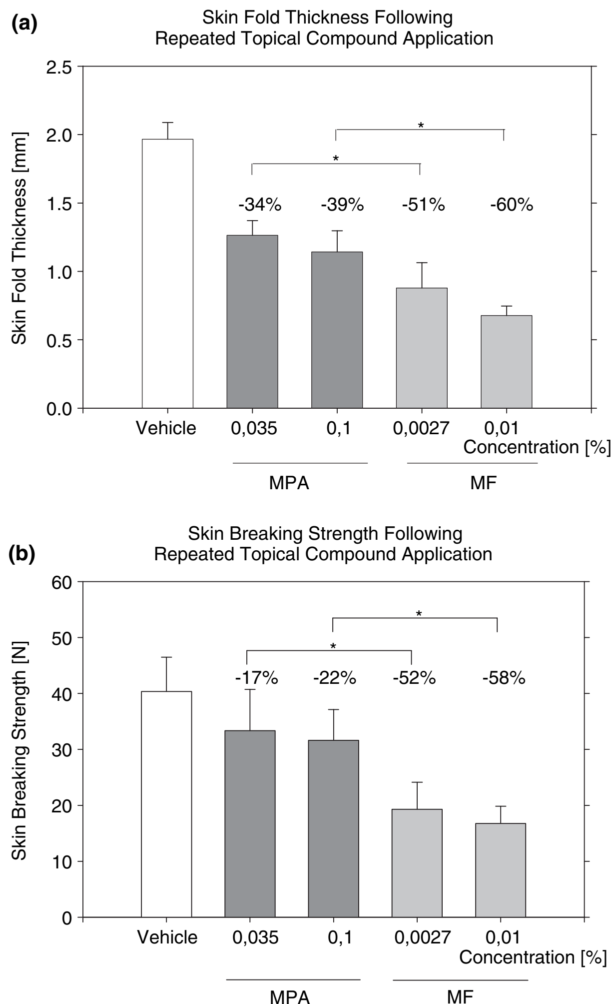

Figure 3. Methylprednisolone aceponate (MPA) has significantly less local side effects relative to mometasone furoate (MF) when topically applied to rat skin. (a) Skin-fold thickness and (b) skin-breaking strength were measured following daily topical application of equipotent ($3 \times ED_{50}$) or equiefficaceous (approximately 80% inhibition of oedema formation in croton oil rat model) concentrations of both compounds compared with vehicle control. Data show mean values ± SD at the end of the experiment on day 19 regarding skin-fold thickness and on day 20 regarding skin-breaking strength. $*P < 0.05$ for MPA versus MF. For all test compounds, $P < 0.05$ versus vehicle control (not shown for clarity reasons). Changes in percent are relative to vehicle control.

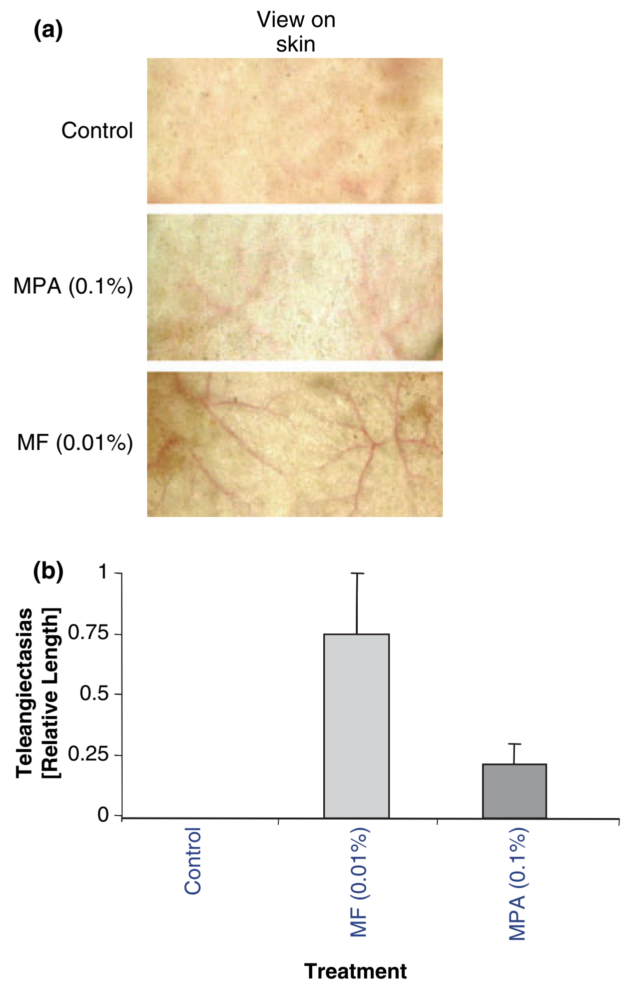

Figure 4. Methylprednisolone aceponate (MPA) causes significantly less telangiectasias compared with mometasone furoate (MF) in *hr/hr* rats. (a) *Hr/hr* rats have been treated topically for 19 days with 0.1% MPA or 0.01% MF, at equieffective concentrations. (b) Length of telangiectasias has been measured by a blinded investigator using the program MetaView, Visitron Inc. Data show mean relative length of telangiectasias ± SD (in pixel) obtained from eight animals per group. $*P < 0.05$ for MPA versus MF, all data significant versus vehicle control.

to skin atrophy, other side effects include osteoporosis, glaucoma and diabetes mellitus induction (28). This study compared MPA with MF regarding nGSR binding affinity; NR-binding affinity was measured relative to the individual receptor's specific ligand and expressed as a ratio of IC_{50} values, with a larger number indicating less GC binding relative to the reference compound. Our data indicate that MPA is more specific regarding NR binding and therefore less likely to induce unwanted side effects compared with MF.

In vitro transactivation assays measuring agonistic and antagonistic effects of MPA and MF on NRs are used as a functional readout of non-specific binding. Again, MPA demonstrates equal or lower nGSR transactivation activity relative to MF. Additionally, Günther et al. (29) reported topical Advantan® application (active ingredient – 0.1% MPA) on intact, stripped and inflamed skin results in plasma levels that did not exceed blank levels corresponding to 1.5 ng MPA Eq/ml (29), which translates to 3 nM MPA. Indeed, the systemic concentration that MPA reaches following topical compound application falls short of the measured EC_{50} (agonism) and IC_{50} (antagonism) values, we determined via transactivation activity assays (Table 2).

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Although GC binding and transactivation were measured *in vitro*, we may employ a theoretical approach for relevance in human skin. The AR is essential for the development and function of male reproductive tissues; additionally, a large number of genes coding for proteins involved in protein folding, trafficking and secretion, metabolism, the cytoskeleton, cell-cycle regulation and signal transduction are regulated via androgens (30). The PR is essential for the female menstrual cycle regulation among other functions (31). The MR is involved in regulating sodium and potassium homeostasis and participates in blood pressure control (32); activation of the MR leads to Na^+ retention and subsequent blood pressure increase (33), which has been implicated with relevance to systemic GC application (34). Considering the broad range of side effects that may arise from nGSR binding, it is advantageous to use a compound with higher specificity for the GR and lower affinity for other NRs. And, although clinical extrapolation in humans must be validated, our findings indicate that increased GR-binding specificity may be linked to an increased TIX. Further studies are needed to assess the clinical relevance of MF's greater nGSR-binding profile and transactivation activity relative to MPA.

Relevant pharmacological *in vivo* concentrations (0.1% for MPA and MF) in man are different than the tested *in vivo* concentrations in rats (0.01% – MPA, 0.001% MF). We analysed equiefficacious and equipotent concentrations to maintain comparability between two compounds that clearly present different potencies. To our knowledge, head-to-head efficacy studies with increasing concentrations of MPA and MF have not been performed which excludes determination of equivalent ED_{50} and ED_{80} concentrations in man. In addition to greater NR-binding specificity, MPA also displays less local side effects compared with MF at equiefficacious and equipotent anti-inflammatory concentrations, which were established utilizing an accepted croton oil-induced dermatitis model in the rat (2). Skin atrophy, tensile strength and telangiectasias are important parameters for measuring the local side-effect profile of topically applied GCs. Following repeated topical compound application, we found a greater reduction in skin thickness in the MF-treated group relative to the MPA-treated group at both equiefficaceous and equipotent concentrations; the same was true of the endpoint measurement. It is worthwhile to note that the decrease in skin thickness observed in our rodent study exceeds the one detected in humans using the same GCs (e.g. 14,18). It has been reported since the 1970s (2,12) that rodent skin is more sensitive towards GC-induced skin atrophy than human skin and, therefore, the magnitude of skin atrophy cannot be translated 1:1 into humans, whereas at least in our experience, the rank order for this side effect is the same in rats and humans. Endpoint skin tensile strength displayed a greater reduction in breaking strength in MF-relative to MPA-treated skin at both concentrations. Finally, MF-treated skin caused more telangiectasias formation relative to MPA-treated skin. From these outcomes, we concluded that MPA induces less local side effects than MF in this rat model.

Our *in vivo* results in the rat demonstrated that MF is more potent than MPA with ED_{50}s of 0.001% vs 0.01% respectively. Although controversial reports on efficacy in humans exist (17,35,36), both MPA and MF are used in 0.1% preparations. As concentrations applied *in humans* are identical for both compounds (i.e. 0.1%), in contrast to the concentrations used *in rats* (10-fold lower concentration of MF versus MPA), it is expected that the differences in side-effect induction observed in this model will be even more relevant in the clinical situation. We therefore speculate that MPA has a greater TIX than MF ($TIX_{MPA} > TIX_{MF}$). The degree to which this conclusion may translate in a clinical setting has yet to be determined. We also conclude that the rat model we utilize here is relevant for the human situation as it mirrors the observations made in human clinical trials so far (18).

Despite significant improvements in topically applied GCs, side effects are not completely excluded in particular after long-term, uncontrolled application leading to an unintentional systemic exposure. Thus, there is still a need for even further improvements. One strategy to limit the systemic effects of GR ligands to the desired anti-inflammatory effects is by searching for dissociated GR-selective compounds. Negative regulation of gene expression by the GR (transrepression) accounts predominantly for its anti-inflammatory action. On the other hand, positive action of the GR through its homodimer binding to discrete nucleotide sequences on effector genes (transactivation) contributes to some of the adverse effects of the hormone. GR ligands that promote the negative regulatory action with reduced positive regulatory functions should therefore show this desired even better therapeutic potential (10,37). Such compounds, called 'selective glucocorticoid receptor agonists (SEGRA)' are currently pursued and may represent the next emerging generation of potent anti-inflammatory agents.

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