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Safer topical treatment for inflammation using 5α-tetrahydrocorticosterone in mouse models

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Dermatitis
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A B S T R A C T

Use of topical glucocorticoid for inflammatory skin conditions is limited by systemic and local side-effects. This investigation addressed the hypothesis that topical 5α-tetrahydrocorticosterone (5αTHB, a corticosterone metabolite) inhibits dermal inflammation without affecting processes responsible for skin thinning and impaired wound healing. The topical anti-inflammatory properties of 5αTHB were compared with those of corticosterone in C57Bl/6 male mice with irritant dermatitis induced by croton oil, whereas its effects on angiogenesis, inflammation, and collagen deposition were investigated by subcutaneous sponge implantation. 5αTHB decreased dermal swelling and total cell infiltration associated with dermatis similarly to corticosterone after 24 h, although at a five fold higher dose, but in contrast did not have any effects after 6 h. Pre-treatment with the glucocorticoid receptor antagonist RU486 attenuated the effect of corticosterone on swelling at 24 h, but not that of 5αTHB. After 24 h 5αTHB reduced myeloperoxidase activity (representative of neutrophil infiltration) to a greater extent than corticosterone. At equipotent anti-inflammatory doses 5αTHB suppressed angiogenesis to a limited extent, unlike corticosterone which substantially decreased angiogenesis compared to vehicle. Furthermore, 5αTHB reduced only endothelial cell recruitment in sponges whereas corticosterone also inhibited smooth muscle cell recruitment and decreased transcripts of angiogenic and inflammatory genes. Strikingly, corticosterone, but not 5αTHB, reduced collagen deposition. However, both 5αTHB and corticosterone attenuated macrophage infiltration into sponges. In conclusion, 5αTHB displays the profile of a safer topical anti-inflammatory compound. With limited effects on angiogenesis and extracellular matrix, it is less likely to impair wound healing or cause skin thinning.

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1. Introduction

Inflammatory skin disease is highly prevalent worldwide: according to estimates published in 2012, eczema affects approximately 230 million people globally, and topical anti-inflammatory glucocorticoids are the most common treatment [1]. However, the therapeutic benefits of these drugs are offset by severe side-effects [2].

Following application of glucocorticoids to the skin, adverse effects manifest locally (skin atrophy and impaired wound healing) and systemically (e.g. osteoporosis, abdominal obesity, and glaucoma) [3]. Skin atrophy is characterised by increased transparency and striae, due to suppression of cell proliferation and inhibition of collagen synthesis, whereas delayed wound healing is attributed to dysregulation of keratinocyte proliferation, fibroblastic activity, and angiogenesis causing delayed formation of granulation tissue [4,5]. These factors restrict the use of more potent glucocorticoids and also the length of treatment, particularly in vulnerable patients such as infants and the elderly. Accordingly, novel drugs or delivery modes have been sought to improve the therapeutic index of topical steroids. This may be achieved by “dissociated glucocorticoids” which invoke trans-repression through the glucocorticoid receptor (GR) without trans-activation [6].

5α-Tetrahydrocorticosterone (5αTHB) is a naturally occurring glucocorticoid metabolite, formed by reduction of the A-ring of...
corticosterone, the principle endogenous glucocorticoid in rodents. Two 5α-reductase isozymes catalyse the rate-determining step in 5αTH synthesis from corticosterone: the type I isozyme is expressed in the liver, kidney and skin; the type II isozyme is expressed principally in the liver, genital tract, prostate and skin. Recent studies have suggested that 5αTHB exhibits a dissociated profile of action, possibly acting through GR. 5αTHB displayed anti-inflammatory properties when administered subcutaneously in mice subjected to thiglycollate-induced peritonitis, but did not induce the chronic adverse metabolic changes (such as increased insulin and body mass) produced by corticosterone [7]. 5αTHB and corticosterone suppressed infiltration of neutrophils into the inflamed peritoneum to a similar extent, but 5αTHB had a lesser effect on macrophage recruitment, suggesting a different profile of action. When infused systemically, 5αTHB demonstrated rapid clearance from the systemic circulation [7], likely limiting its oral use, but the same characteristic would be beneficial in topical treatments in which systemic effects are unwanted.

This investigation addressed the hypothesis that topical 5αTHB can effectively reduce skin inflammation through a GR-dependent mechanism, but with limited effects on processes such as angiogenesis and collagen deposition, the inhibition of which underpin most common problems associated with topical glucocorticoid therapy. The efficacy of topical 5αTHB application for treating skin inflammation was compared with that of corticosterone in vivo using croton oil-induced dermatisis and the role of GR was assessed by pre-administration of the GR antagonist mifepristone (RU486). This is a steroid-responsive model of dermal inflammation, driven primarily by neutrophil accumulation, commonly used for testing anti-inflammatory treatments for irritant dermatitis [8,9]. An in vivo model of sponge implantation allowed comparison of the effects of corticosterone and 5αTHB on inflammation, angiogenesis and collagen deposition.

2. Materials and methods

2.1. Materials

Chemicals were from Sigma Aldrich (Poole, UK) unless otherwise stated. Steroids (corticosterone, 5αTHB and RU486) were from Steraloids (Newport, RI, USA).

2.2. Animal welfare, models and ethical statements

Male mice (C57BL/6, 8–10 week old) were from Harlan Laboratories (Sharlrow, UK). Animals were allowed to acclimatise for one week prior to experimentation, maintained under controlled conditions of light (lights on 0700–1900 h) and temperature (18–20 °C) and allowed free access to standard chow (Special Diet Services, Witham, UK) and drinking water. All experimental procedures were performed under UK Home Office guidelines.

2.2.1. In vivo model of irritant dermatitis

Mice were treated on the inner surface of the right ear with either croton oil only (CO mixture: 10 μL, 3% v/v in ethanol and isopropyl myristate 30:5) or with solutions of corticosterone or 5αTHB diluted in the CO mixture; the left ear was untreated. The EC₅₀ dose of corticosterone to reduce swelling was determined performing dose-response experiments (0.3–30 μg; 6 h, n = 4/group; 24 h, 8–12/group) in which a “non-linear log(agonist)” versus response (three parameters) regression curve was fitted using GraphPad 6 software (La Jolla, CA, USA). Subsequently, the efficacy of 5αTHB was compared using doses corresponding to 1, 3 and 5 times the EC₅₀ of corticosterone after 6 h (corticosterone, 10 μg; 5αTHB, 10–50 μg; n = 4–8/group) or 24 h (corticosterone, 5 μg; 5αTHB, 5–25 μg; n = 8–12/group). Mice were culled by asphyxiation with carbon dioxide and ears excised and wet weighed. Inflammatory swelling was evaluated as the wet weight difference between treated and untreated ears, and the effect of steroids is presented as a percentage of the response to CO alone (mean weight difference = 100%).

2.2.2. In vivo model of irritant dermatitis and treatment with RU486 in adrenalectomised mice

Male mice (n = 6–11/group) were injected, prior to treatment with steroids, with the GR antagonist RU486. Adrenalectomised mice were used to avoid the influence of increased physiological levels of glucocorticoids due to stress responses.

2.2.2.1. Surgery. Bilateral adrenalectomy surgery was performed through dorsal incisions, under isoflurane anaesthesia (Merital, Harlow, UK). After surgery, animals were maintained on 0.9% saline drinking water to maintain fluid and electrolyte balance and allowed to recover for one week before subsequent intervention.

2.2.2.2. RU486 treatments. RU486 (0.5 mg/mouse; 25 μL, 20 mg/mL in ethanol (vehicle)) was injected subcutaneously into mice 15 min prior to any topical treatment. Contact irritant dermatitis was induced and steroid treatment applied (24 h) as described in Section 2.2.1.

2.2.3. In vivo model of inflammatory angiogenesis

An autoclaved cubic polyurethane sponge (~0.5 cm³; grade xe1700w, Caligen Foam Ltd., Accrington, U.K.) was implanted sub-cutaneously on each flank of mice anaesthetised with isoflurane (Merial, Harlow, UK). For the delivery of steroids, one silastic pellet (Silastic 20 medical grade, Dow Corning®, Midland, USA) impregnated with vehicle (silastic only), corticosterone (3 mg/pellet) or 5αTHB (3 or 15 mg/pellet) was inserted in each sponge. Validation of the preparation and release properties of these pellets has been reported [10]. Each animal in the treatment groups had an intervention-impregnated sponge (corticosterone or 5αTHB) implanted on the right side, and a vehicle sponge on the left. Animals in the control group had vehicle sponges implanted on each side. 21 days after surgery, mice were euthanized, sponges excised and pellets removed. Sponges were bisected and one half was preserved in 10% formalin (in PBS) and the other immersed in RNA later solution.

2.3. Laboratory analysis

2.3.1. Histological analysis

Paraffin-embedded ear tissue and sponge slices (5 μm) were rehydrated and stained with haematoxylin and eosin (H&E) or with Picrosirius red solution (PRS) for collagen content analysis (sponges only), prior to dehydration and mounting. Images were captured using the software QCapture Pro 7 (Qlaiming, Canada). On H&E stained ear sections the width of the dermis (in μm) and cell infiltration (number of nuclei) were assessed as further measurements of swelling and inflammation at both sides of the central cartilage layer. For each parameter three different areas were analysed and the mean value calculated.

2.3.2. Quantitation of neutrophils by myeloperoxidase activity

Myeloperoxidase (MPO) activity in ear tissue was quantified using a fluorometric detection kit (ENZO Life Sciences (Exeter, UK)).
2.3.3. Quantification of angiogenesis, macrophage infiltration and collagen content in sponges

For each sponge section vessels were counted in three different areas at magnification 100X. Vessels in sections stained with H&E were recognized by their round appearance and the presence of erythrocytes in the lumen. Vessels identified in sections immunostained for CD31 and αSMA marker were counted. The numbers of newly formed vessels and vessels positive for either CD31 or αSMA are represented as percentage of the vehicle group (mean value = 100%). Macrophages were counted as the number of cells positive for the marker F4/80 and expressed as a proportion of the total number of cells (blue nuclei; magnification 200X). PRS staining was quantified calculating the number of red pixels at a magnification of 100X using ImageJ software (NIH, USA).

2.3.4. Immunostaining for CD31, αSMA and F4/80 in sponges

Immunostaining of sponge slices was performed using a Leica Staining Robot with robotic antigen retrieval for F4/80 using trypsin solution (0.5 mg/mL in PBS, 10 min, 37 °C), or for αSMA manual antibody retrieval using a pressure cooker under standard conditions (citrate NCL pH 6 buffer); antigen retrieval was not performed for CD31. For F4/80 and αSMA, samples were blocked (5 min) with peroxidase solution from the Leica Refine Detection Kit (Leica Biosystems, Milton Keynes, UK) followed by serum block (30 min; F4/80, Impress Kit was used, which included a hydrogen peroxide block (5 min), primary antibody (30 min; F4/80 1:300 (eBio-Mouse Abcam Kit (Cambridge, UK)). Thereafter, samples were incubated with primary antibody (30 min; 5 min, F4/80 1:300 (eBio-Mouse Abcam Kit (Cambridge, UK))). Thereafter, samples were incubated with primary antibody (30 min; 5 min, F4/80 1:300 (eBio-Mouse Abcam Kit (Cambridge, UK))).

2.3.5. Total RNA extraction and PCR

Total RNA was extracted from ears and sponges using the RNeasy Fibrous Tissue Mini Kit, and first strand cDNA synthesis was performed using the Quantitect Reverse Transcription kit (Qiagen Ltd, West Sussex, UK). Real-time quantitative PCR was performed using a LightCycler® 480 (Roche Diagnostics, Mannheim, Germany) with primers (6 pmol each, Table 1), corresponding 5′-labelled probe (UPL, 2 pmol) and LightCycler®480 Probes Master [11]. Data were normalized for the mean of the transcript abundance of two housekeeping genes, TATA-binding protein and glyceraldehyde 3-phosphate dehydrogenase, the abundances of which did not differ between groups, and represented as percentage of the respective control group whose mean value was set to 100%.

2.4. Data analysis

All data were analysed using GraphPad Prism6 software and presented as mean ± SEM, using statistical tests as indicated. Significant differences were reported when p < 0.05. For histological analysis, the operator was blinded to treatment.

3. Results

3.1. 5xTHB reduces croton oil-induced inflammation with a different time course than corticosterone

Increasing concentrations of corticosterone reduced croton oil-induced ear swelling in a dose-dependent manner after 6 and 24 h (Fig. 1a, b), with EC50 doses of 10 μg at 6 h and 5 μg at 24 h. 5xTHB reduced swelling at 24 h (EC50 dose: 25 μg) but not at 6 h (Fig. 2a–d). Only samples treated with the highest doses of 5xTHB (50 μg at 6 h and 25 μg at 24 h) were subject to further analysis. Histological and biochemical analyses showed that croton oil-induced swelling was accompanied by enlargement of the dermal layer at both 6 and 24 h (Fig. 2a–d). However, 5xTHB (50 μg) reduced swelling at 24 h (Fig. 2e, f).

The involvement of GR in mediating the anti-inflammatory effects of corticosterone and 5xTHB was assessed by treating adrenalectomised mice injected with the GR antagonist RU486. Neither adrenalectomy nor ethanol nor RU486 injection influenced the extent of CO-induced swelling (Fig. 3a). Injection of RU486 attenuated the anti-inflammatory effect of corticosterone but not that of 5xTHB (Fig. 3b).

Table 1

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At 24 h, croton oil increased all transcripts characterising inflammatory responses, altered vascular permeability and extracellular matrix (ECM) regulation, with the exception of AnxA1 (Fig. 4a–c). Corticosterone reduced abundance of Ifn-γ, Vegf, Pecam1, Icam1, Icam2, P- and L-selectin, Mmp9, Keratin6 and Col4a1 and increased abundance of AnxA1 compared to the group receiving croton oil alone. 5αTHB caused similar changes to those of corticosterone, with the exception of Icam2, L-selectin, Mmp9 and

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Keratin6 which were not affected; additionally, 5αTHB increased transcripts of the anti-inflammatory gene Dusp1 and decreased those of Vcam1.

3.2. 5αTHB and corticosterone have discrepant effects on de novo angiogenesis in sponges

Sponges containing vehicle pellets (V) were bright red, suggesting extensive vascularization, whereas those with corticosterone pellets (3 mg) were pale (similar to pre-implantation sponges) indicating less vascularization (Fig. 5a). Sponges with 5αTHB (3 mg) had a similar appearance to vehicle-treated sponges, whereas those with 15 mg 5αTHB were somewhat paler, although the lack of vascularization was not as extensive as that shown by sponges containing corticosterone.

Vessels were identified by H&E staining (Fig. 5b), and immunoreactivity for CD31 (Fig. 5c) and αSMA (Fig. 5d) associated with vascular structures. Quantification showed that corticosterone dramatically reduced vessel density whether assessed by H&E (Fig. 5e), CD31 (Fig. 5f) or αSMA (Fig. 5g) staining. In contrast, an equivalent dose (3 mg) of 5αTHB in sponges had little effect on vascular density (Fig. 5e, f, g). The higher dose (15 mg: equivalent in anti-inflammatory model) reduced vascular density assessed by H&E (Fig. 5e) and CD31 (Fig. 5f) staining but less dramatically than with corticosterone. Strikingly, 5αTHB had little effect on the density of αSMA positive vessels (Fig. 5g). Macrophage infiltration of sponges was observed (Fig. 6a, c) and was reduced following treatment with corticosterone or 5αTHB, although this did not achieve significance at the lower dose of 5αTHB (Fig. 6c). Collagen staining (Fig. 6b, d) was lower in sponges treated with corticosterone, compared with vehicle-treated controls, whereas 5αTHB had no effect at either concentration.

Corticosterone treatment was associated with increased transcripts for Vegfα and Vcam1 whereas transcripts for Vegf2, Icam1, Pecam1 (CD31), VE-cadherin, E-selectin and Acta2 (αSMA) were decreased (Fig. 7a). In contrast, 5αTHB only decreased transcripts of Pecam1. Corticosterone reduced the transcript abundance for the inflammatory genes Tnfs1, Il1β and Ifny; but had no effect on Mcp1 or Il6 (Fig. 7b). In contrast, neither concentration of 5αTHB reduced any of the gene transcripts, with the lower concentration (3 mg) in fact increasing the abundance of transcripts for Mcp1 (Fig. 7b). In sponges containing corticosterone, transcripts for two matrix metalloproteinases (Mmp2; Mmp10) and four collagen types (Cola1a1, Cola1a2, Cola3a1, Cola4a1) were reduced compared to vehicle (Fig. 7c) but tissue inhibitor of metalloproteinases (Timp1) and matrix metalloproteinase Mmp9 was unchanged. In contrast, 5αTHB (3 mg & 15 mg) had limited effects, only decreasing Col1a2.

4. Discussion

Anti-inflammatory properties, but a safer systemic side effect profile, of 5αTHB, an A-ring reduced metabolite of corticosterone, have been demonstrated previously in vivo in a murine model of thioglycolate-induced peritonitis [7]. This investigation addressed the hypotheses that 5αTHB has advantages over conventional glucocorticoids as a topical treatment for irritant dermatitis, and that its actions are mediated through GR. 5αTHB was demonstrated to be an effective topical anti-inflammatory agent in an in vivo model of irritant dermatitis induced by croton oil, with effects mainly on pathways regulating vascular permeability to cell infiltration rather than suppression of pro-inflammatory markers. However, experiments using the GR antagonist RU486 suggested these effects may not be mediated by occupation of GR. Furthermore, in comparison with corticosterone, use of 5αTHB may be associated with fewer adverse local effects (less inhibition of angiogenesis and collagen deposition).

The direct suppression of inflammation by topical 5αTHB was assessed using croton oil-induced irritant dermatitis, a well-characterised, steroid-responsive model, used extensively in the pharmaceutical industry [6,12–14]. Croton oil promotes the release of cytokines and chemokines from keratinocytes and other cells, stimulating in turn the release of pro-inflammatory cytokines such as IL1β and IL6 from epidermal and dermal cells, causing swelling and infiltration of leukocytes [15–18]. The predominant effect at the early stages of the process is tissue oedema which peaks 6 h-post treatment; this phase is followed by cell infiltration (mainly neutrophils) which is at its highest between 12 and 24 h [19–22]. This time course of changes was recapitulated here: at 6 h croton oil produced a marked swelling accompanied by cell infiltration and increased MPO activity, whereas at 24 h swelling persisted but was reduced, while cell infiltration and MPO activity increased further.

This model has been used extensively to test novel anti-inflammatory drugs, including glucocorticoids (e.g. dexametha-
Fig. 4. Corticosterone (B) and 5α-tetrahydrocorticosterone (5αTHB) had limited but similar effects on a variety of gene transcripts in mouse ears in a model of croton oil (CO)-induced irritant dermatitis after 24 h. Real-time PCR analysis of transcripts for (a) inflammatory, (b) vascular and (c) extra-cellular matrix (ECM) genes in control ears (CTRL) or ears treated for 24 h with either CO alone or together with B 5 μg or 5αTHB 25 μg. Data (mean ± SEM) were analysed with one-way ANOVA followed by Tukey’s post hoc test; n = 10/group; p < 0.05, # = vs CO, \* = vs CTRL, € = B vs 5αTHB.

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Fig. 5. Corticosterone (B) but not 5α-tetrahydrocorticosterone (5αTHB) dramatically reduced vessel number in a model of in vivo angiogenesis. (a) Representative macroscopic pictures of sponges retrieved from mice after 20 days in situ, and containing either vehicle pellets (V) or pellets loaded with either B (3 mg) or 5αTHB (3 mg & 15 mg) as indicated. Representative microscopic images (magnification = 100x) of sections of sponges stained with (b) haematoxylin and eosin (H&E) or immunostained for (c) the endothelial marker CD31 or (d) for the smooth muscle cell marker αSMA. Black arrows point either to vessels recognisable by H&E staining or positive for the marker indicated and magnified in the corresponding inset (400x) of each image; * = examples of vessels undergoing sprouting angiogenesis. Quantification of newly formed vessels in sponges containing either vehicle (V), B or 5αTHB and stained with (e) H&E or positive for (f) CD31 or (g) αSMA. Numbers on the x axis refer to the amount (mg) of steroids in each sponge; data (mean ± SEM) were analysed with one-way ANOVA followed by Tukey’s post hoc test; n = 8–12 mice/group; p ≤ 0.05, # = vs V, * = vs B, £ = 5αTHB 3 mg vs 15 mg.

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sone, betamethasone and hydrocortisone) [21,23–26]. Here, the effects of 5α-THB on skin inflammation were compared with those of corticosterone, the active rodent glucocorticoid. Previous reports of corticosterone efficacy were not available but our data suggest that at 6 h it is twice as potent as one of the most frequently employed topical glucocorticoids, hydrocortisone[21,27]. Notably, the dose-dependent reduction of dermal inflammation by 5α-THB exhibited a slower time course than corticosterone with no effect at 6 h. Significant suppression of inflammation was, however, achieved at 24 h, albeit requiring a 3–5 fold higher dose than corticosterone to match efficacy. This suggests that 5α-THB may predominantly target cell recruitment, which is prominent at 24 h post CO-treatment, with neutrophils believed to be the most abundant cell type present [19–22]. Analysis of MPO activity showed that both steroids reduced neutrophil infiltration; interestingly, this was suppressed more effectively by 5α-THB than by corticosterone at 24 h. The lack of response to 5α-THB to reduce swelling at 6 h was accompanied by a failure to reduce cell number.

Experiments with the GR antagonist RU486 suggested that the anti-inflammatory effect of B on swelling is mediated by GR. However, this seems not to be the case for 5α-THB. The fact that the anti-inflammatory actions of 5α-THB were not antagonised by RU486 was unexpected, albeit in keeping with its different time course of action to corticosterone. 5α-THB is a glucocorticoid metabolite, Fig. 6. Corticosterone (B) and 5α-tetrahydrocorticosterone (5αTHB) decreased macrophage infiltration in sponges but only B reduced collagen content. Representative microscopic images (magnification 100x) of sections of sponges stained for (a) the macrophage marker F4/80 or for (b) collagen with Picrosirius red and containing either vehicle (V), B (3 mg) or 5αTHB (3 and 15 mg) as indicated. Black arrows in (a) point to cells expressing F4/80; the blue arrow in the inset (1000x) identifies the nucleus of a cell negative for F4/80. Quantification of (c) cells positive for F4/80 and (d) collagen content in sponges containing either V, B or 5αTHB; numbers on the x axis refer to the amount (mg) of steroids in each sponge. Data (mean ± SEM) were analysed with one-way ANOVA followed by Tukey’s post hoc test; n = 8–12 mice/group; p ≤ 0.05, # = vs V, * = B vs 5αTHB.
previously shown capable of displacing dexamethasone from rat GR in vitro [28]. RU486 acts as a GR antagonist by binding to the ligand-binding domain, blocking the recruitment of essential GR co-activators [29,30], and if 5αTHB does operate through GR it does so by an unconventional mechanism, perhaps through allosteric means. Further genetic and pharmacological studies are now required to elucidate the mechanism of action of 5αTHB.

To start to address this, the profile of action of 5αTHB was explored further through expression profiling and compared with responses produced by corticosterone. At 24 h, the impact of 5αTHB on expression of genes involved in regulation of inflammation and vascular homeostasis in the CO model was strikingly similar to those of corticosterone. A noted difference was in the upregulation of Dusp1 by 5αTHB in contrast to corticosterone. DUSP1 is a negative regulator of the MAPK signal transduction pathway, and is increased in response to inflammatory and irritant stimuli, leading to decreased production of cytokines, chemokines and adhesion molecules [31,32]. A glucocorticoid-mediated increase in its protein abundance has been reported previously in many cell types [33–37]. DUSP1 is also upregulated by non-steroidal anti-inflammatory compounds [32] and by new selective modulators of GR [38]. 5αTHB and corticosterone also increased transcripts of the anti-inflammatory gene AnxA1 (lipocortin). Lipocortin was originally described as being responsible for the anti-inflammatory properties of glucocorticoids [39], again diminishing neutrophil recruitment by suppressing adhesion to endothelial
cells [39]. Both corticosterone and 5xTHB strongly suppressed many factors involved with tissue permeability and cell recruitment. The effects of 5xTHB on expression of ECM proteins were less marked than those of corticosterone, whereas both lacked effects on transcription of most collagen genes, only decreasing Col4a1 transcripts. Type IV collagen is one of the minor components of the skin (less than 5%) [5], mainly forming the basement membrane; as a consequence both steroids are likely to have little impact on skin thickness of croton-oil treated ears after 24 h. However accurate measures of skin thickness cannot be made in the inflamed skin in vivo. Previous in vitro microarray studies on cultures of primary human keratinocytes showed that dexamethasone, a glucocorticoid known to cause skin thinning, decreases transcripts of primary human keratinocytes showed that dexamethasone, a glucocorticoid known to cause skin thinning, decreases transcripts of Pecam1, Keratin6, FilaminA and Col4a1 after 24 h incubation [40], a profile similar to that of corticosterone in our model.

Two of the major side effects of topical anti-inflammatory glucocorticoids are skin thinning and delayed wound healing, requiring careful assessment of potential adverse effects for any proposed topical steroid therapy. Angiogenesis and collagen turnover are important factors in skin homeostasis, with angiogenesis particularly important for dermal healing [41]. Following the demonstration that cortisone inhibits angiogenesis in vitro, a number of “angiostatic steroids” have been identified [42]. These include 5β-tetrahydrocortisol, one of the A-ring reduced metabolites of cortisol (the major glucocorticoid in humans). Here the impact of 5xTHB on angiogenesis, inflammation and collagen deposition was studied in response to implantation of subcutaneous sponges [43]. This model is steroid sensitive [44] and very accurate measures of skin thickness cannot be made in the inflamed skin in vivo. Previous in vitro microarray studies on cultures of human keratinocytes showed that dexamethasone, a glucocorticoid known to cause skin thinning, decreases transcripts of Pecam1, Keratin6, FilaminA and Col4a1 after 24 h incubation [40], a profile similar to that of corticosterone in our model.

In conclusion, 5xTHB, despite reducing macrophage infiltration, did not reduce expression of any of the transcripts analysed and, indeed, increased abundance of transcripts of Mcp1. A similar induction of Mcp1/MCP1 promoted vessel growth in infarcted hearts in mice [54], suggesting this may be a compensatory mechanism in response to an ischemic event. In previous studies of peritonitis 5xTHB also suppressed inflammatory cell recruitment, but with a more profound effect on neutrophils compared with macrophages [7].

In conclusion, 5xTHB, despite reducing macrophage infiltration, did not reduce expression of any of the transcripts analysed and, indeed, increased abundance of transcripts of Mcp1. A similar induction of Mcp1/MCP1 promoted vessel growth in infarcted hearts in mice [54], suggesting this may be a compensatory mechanism in response to an ischemic event. In previous studies of peritonitis 5xTHB also suppressed inflammatory cell recruitment, but with a more profound effect on neutrophils compared with macrophages [7].
effects, suggest they may have potential as a novel topical anti-inflammatory treatment and careful dissection of its mechanism of action is now required.

Conflicts of interest

RA and BRW are inventors on a relevant patent held by the University of Edinburgh.

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