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INVESTIGATIVE REPORT

Dose-response Effects of Tri-iodothyroacetic Acid (Triac) and Other Thyroid Hormone Analogues on Glucocorticoid-Induced Skin Atrophy in the Haired Mouse

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Thyroid hormones have an influence on the connective tissue biology of the skin and, theoretically, topically applied thyroid hormones or hormone analogues could have a stimulatory effect on collagen synthesis. In this investigation the effect of topical tri-iodothyroacetic acid (Triac) and other thyroid hormone analogues were tested for their effect in preventing betamethasone-induced skin atrophy in the normal haired mouse. Triac, tri-iodoproprionic acid (Triprop) and the synthetically developed thyroid hormone analogue KB-026 and 2 different Triac cream formulations were applied along with betamethasone on shaved mouse skin. Triac in daily doses of 1 nmol/cm^2 and higher was able to block the betametbasone-induced skin atrophy in mice skin. In high doses, Triprop and KB-026 also had a blocking effect. Triac alone had a stimulatory effect on dermal thickness. This study indicates that thyroid hormone analogues may be used to prevent corticosteroid-induced skin atrophy. ***Keywords: animal model; skin thickness; normal haired mouse.***

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Collagen is the most abundant protein of skin. During the ageing process, collagen level and biosynthetic activity undergo marked changes. In addition, there are alterations in collagen turnover in several skin diseases and upon treatment with various drugs. For example, it has been demonstrated that in human skin *in vivo*, topical glucocorticoid treatment decreased collagen levels (1). Furthermore, in the clinical setting, treatment with glucocorticoids, topical as well as systemic, is frequently associated with a variety of side effects on the skin such as epidermal atrophy, dermal thinning, striae and telangiectasia.

The impact of increased or decreased blood levels of thyroid hormones (TH) on skin status is well known (2, 3). Excessive amounts, as in thyrotoxicosis, are accompanied by cutaneous alterations due to increased dermal blood flow as a consequence of increased metabolic rate and decreased systemic vascular resistance and are thus not attributable to a direct action of TH on cells in the skin (2–5). However, it is also known that the connective tissue biology of the skin is sensitive to alterations in thyroid hormone status (3) and the presence of intracellular receptors (TRs) for thyroid hormones in human skin, in both dermal and epidermal parts, have been demonstrated (6–10).

Information concerning the mechanism of action of TH in fibroblasts, especially in regulation of collagen production, is limited. In one study by Rycker et al., thyroid hormone administration diminished collagen production in cultured fibroblasts (9). Although a vast number of studies on thyroid hormone action in organs such as liver, heart, pituitary, brain and adipose tissue have been performed, no studies have been undertaken to investigate the activity of topical TH in glucocorticoid-treated skin. Moreover, as TRs are part of a larger superfamily of nuclear receptors, which comprises the receptors for glucocorticoids, retinoic acid and vitamin D, which are targets for many topical pharmaceuticals, this has motivated studies on the potential role of topical treatment with TH or TH analogues for dermal disorders (4, 10).

Earlier studies in the hairless C3H mouse, which are homozygotic mutants in the recessive *hr* locus, have shown that treatment once a day with betamethasone in an isopropanol/water vehicle induced a substantial thinning of the skin in a short period of time (10). Skin thickness was assessed using 3 different methods; skinfold thickness with calipers, sonography and histometry (using a microscope with an ocular scale) of stained sections from punch biopsies, all of which provided consistent and essentially the same results (10, 11). Since this model had been shown to be a robust and relatively rapid way to study the direct effects of a ligand for a nuclear receptor (the vitamin D analogue KH1060) in intact skin, we decided to use a similar but haired mouse model to test the activity of TH analogues.

The aim of our study was, in a simple model, to evaluate whether Triac and other TH analogues could prevent bethamethasone-induced thinning of normal mouse skin. The decision to use haired mice (after shaving)

obliged us to perform each experiment with mice of the same age and to exercise care when comparing experiments between groups delivered on different occasions.

MATERIAL AND METHODS

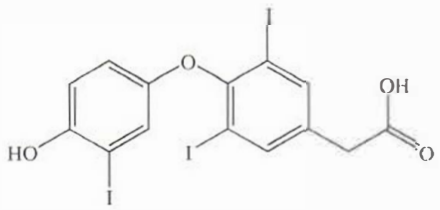
The study outline and procedure were approved by the animal ethics committee of Gothenburg University.

Animals

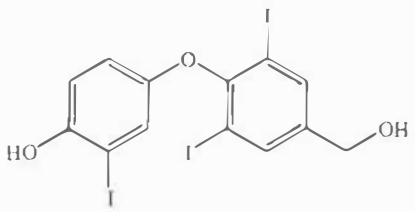
Normal healthy Balb C, male mice (25–30 g) (Bomholtegaard Breeding and Research Centre, Denmark) were housed in cages, with 4 to 5 animals in each group. Standard pellets and water were provided *ad libitum*.

Drugs

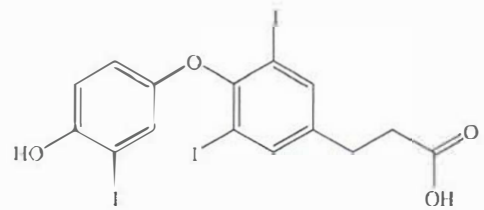
Betamethasone was obtained from Sigma (St Louis, USA). Triac and the other TH analogues KB-026 and tri-iodoproprionic acid (Triprop) (Fig. 1) were obtained from Karo Bio (Huddinge, Sweden). Essex cream was obtained from Schering Plough (New Jersey, USA). The solvent used in the experiments was isopropanol, 50% in water. The various concentrations of betamethasone and the TH analogues were obtained by mixing them with the solvent. A fixed daily dose of 100 µl of a 0.2 mM solution corresponding to 2.5 nmol/cm^2 skin of betamethasone was used in all groups treated with betamethasone. Triac cream was made by mixing Triac with Essex cream to obtain a concentration of 0.01% and 0.03% of Triac in the cream.



Triac



KB026



Triprop

Fig. 1. Chemical structure of tri-iodothyroacetic acid (Triac), KB-026 (a synthetic thyroid hormone analogue) and tri-iodoproprionic acid (Triprop).

Experimental design

The hair of a normal mouse was shaved once daily on the back on a 2×4 cm area using a standard hair shaver. All animals in the experimental groups were shaved at the same time. Aliquots (100 µl) of each of the test drug solutions were applied once daily for 7 days using a micropipette and spread with a gloved finger on the shaved area.

The test area was visually assessed on day 7 and the animals were then killed by barbiturate overdose. Immediately afterwards, the skinfold thickness was measured with a caliper. A 3-mm punch biopsy was taken, fixed in formalin and cut into 6 µm thin sections with 3 sections on each glass and 5 glasses for each treatment group. The skin sections were stained with hematoxylen-eosin staining and Van Gieson for collagen staining. The thickness of the dermis (in mm) was measured blindly with 5 measurements, one from each of the 5 best sections, and the mean of these 5 measurements was calculated and taken as the value. The epidermis was not included because it is difficult to determine exactly where the stratum corneum starts.

Three different sets of experiments were performed:

1. The effect of various concentrations of Triac on betamethasone-induced skin atrophy. The details are shown in Table I.

2. The effect of various concentrations of Triac and Triac cream on betamethasone-induced skin atrophy. As in experiment 1, the effect of Triac after betamethasone treatment was tested. In addition, one group received topical treatment with Triac alone. In 3 separate groups of mice, betamethasone was applied on the skin on the backs of the mice and 10 min later 0.05 g of a cream (Essex^R, Schering Plough, New Jersey, USA) containing 0.01% of Triac, 0.03% of Triac or placebo cream was applied.

3. The effect of various concentrations of Triac cream, KB-026 and Triprop on betamethasone-induced skin atrophy. Triac in Essex cream was tested again. In an additional 8 groups of mice, combinations of betamethasone and KB-026 or Triprop were studied.

Statistics

Wilcoxon's rank sum test was used to compare differences in skinfold thickness and differences in dermal thickness between the histopathologic sections.

RESULTS

The effect of topically applied Triac on betamethasone-induced skin atrophy was studied in three partially overlapping experiments (Table I). Experiment 2 was similar to experiment 1, except that the effect of Triac alone and the effect of Triac in a cream formulation were also investigated. In experiment 3, the effects of 2 other TH analogues (KB-026 and Triprop) were also investigated.

Visual assessment

No visual assessments were carried out in the first experiment. For the rest of the experiments, no visual signs of skin atrophy or skin irritation were observed in any of the groups.

Table I. *Dermal thickness as result of treatment with different thyroid hormone analogues: Triac (tri-iodothyroacetic acid) (in isoporopanol/water or in Essex-cream), KB026 and Triprop (tri-iodoproprionic acid) on betamethasone-induced skin thinning in a mouse model*

Vehicle used	Daily topical BM-dose (nmol/cm²)	Daily topical TH-analogue dose (nmol/cm²)	Thickness of dermis (mm)*		
			Mean	±SD	*p*-value
Experiment 1 (n=5)					
IsoPr 50%	2.5	–	0.37	0.01	
IsoPr 50%	2.5	Triac 0.01	0.38	0.02	
IsoPr 50%	2.5	Triac 0.1	0.45	0.02	
IsoPr 50%	2.5	Triac 1	0.50	0.02	<0.05
IsoPr 50%	2.5	Triac 10	0.50	0.02	<0.05
IsoPr 50%	2.5	Triac 100	0.50	0.02	<0.05
IsoPr 50%	–	–	0.50	0.01	<0.05
Experiment 2 (n=4)					
IsoPr 50%	2.5	–	0.39	0.02	
IsoPr 50%	2.5	Triac 0.01	0.40	0.02	
IsoPr 50%	2.5	Triac 0.1	0.40	0.02	
IsoPr 50%	2.5	Triac 1.0	0.50	0.03	<0.05
IsoPr 50%	2.5	Triac 10	0.58	0.03	<0.05
IsoPr 50%	–	Triac 10	0.79	0.05	<0.05
Essex	2.5	–	0.38	0.02	
Essex	2.5	Triac 1.0	0.49	0.02	<0.05
Essex	2.5	Triac 3.0	0.55	0.03	<0.05
IsoPr 50%	–	–	0.58	0.01	<0.05
Experiment 3 (n=4)					
IsoPr 50%	2.5	–	0.42	0.05	
Essex	2.5	–	0.40	0.01	
Essex	2.5	Triac 1.0	0.50	0.03	<0.05
Essex	2.5	Triac 3.0	0.56	0.04	<0.05
IsoPr 50%	2.5	KB026 0.01	0.41	0.01	
IsoPr 50%	2.5	KB026 0.1	0.44	0.01	
IsoPr 50%	2.5	KB026 1.0	0.48	0.03	
IsoPr 50%	2.5	KB026 10	0.50	0.01	<0.05
IsoPr 50%	2.5	Triprop 0.01	0.42	0.03	
IsoPr 50%	2.5	Triprop 0.1	0.45	0.04	
IsoPr 50%	2.5	Triprop 1.0	0.48	0.02	
IsoPr 50%	2.5	Triprop 10	0.48	0.03	
IsoPr 50%	–	–	0.54	0.02	<0.05

Mean value and standard deviation of 5 low-power fields. The *p*-value <0.05 denotes significant difference (of 95% probability) as compared to thickness in the positive control (Betamethasone alone; first group in each experiment).
BM: betamethasone; TH: thyroid hormone.

Skinfold thickness measurements

No measurements were done in the first experiment. For the other experiments, the only statistically significant difference in skinfold thickness was found between Triac alone and betamethasone alone (35% increase) ($p<0.05$). Thus this method is only able to detect rather large differences in thickness.

Histopathology

The results are shown in Table I. In the control group the mean thickness of dermis was 0.50 mm. Betamethasone alone induced a reduction in dermal thickness of 0.13 mm (Fig. 2*a*). The same dermal thickness as that in the negative control group was seen in the groups of mice treated with a combination of betamethasone and Triac in concentrations of 1 nmol/cm² and higher (Fig. 2*b*). Statistically, this difference was significantly different ($p<0.05$) compared to the group treated with betamethasone alone.

In experiment 2, the effect of Triac alone (Table I) was studied. Triac had a stimulatory effect on dermal thickness with an increase from 0.60 mm to 0.82 mm ($p<0.05$) (Fig. 2*c*). Both concentrations of Triac in a cream (Essex^R) vehicle prevented betamethasone-induced skin atrophy, and the results seen with the various concentrations of Triac were comparable with those seen in experiment 1.

KB-026 in a dosage of 10 nmol/cm² had an inhibitory effect ($p<0.05$) on the betamethasone-induced skin atrophy (Experiment 3, Table I). Again, both of the Triac cream formulations prevented betamethasone-induced skin atrophy ($p<0.05$).

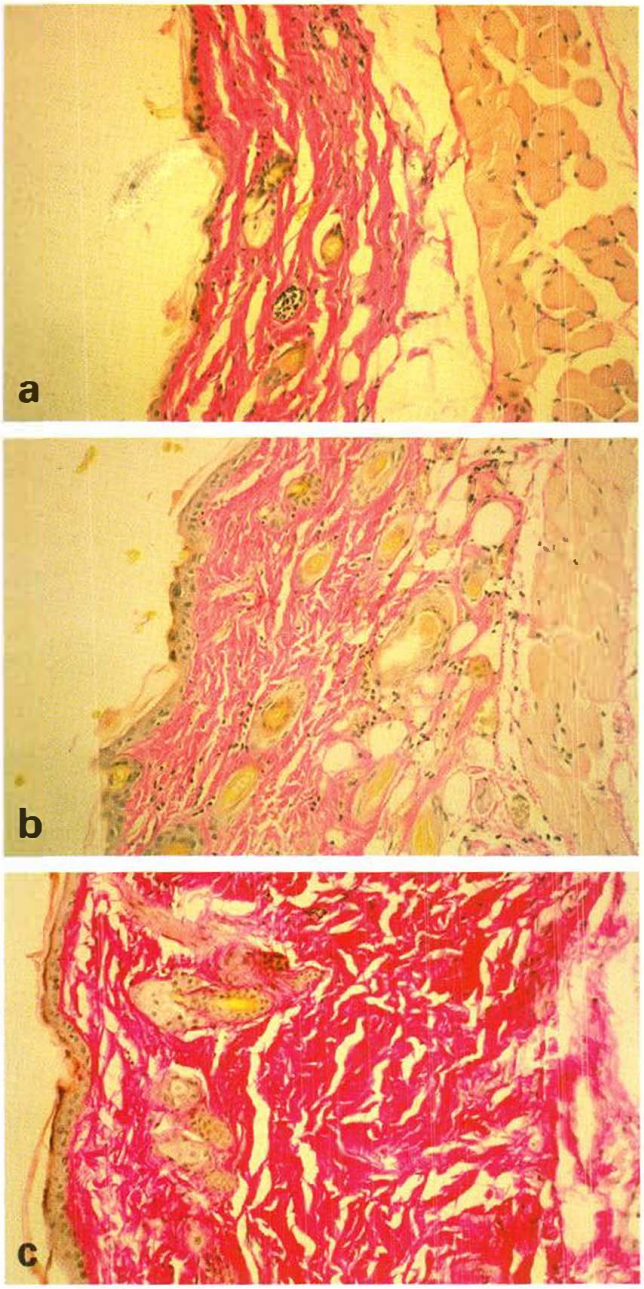


Fig. 2. Skin histology showing the skin thickness after 7 days of various treatments (van Gieson × 200). Betamethasone (2.5 nmol/cm^2) (a), a combination of betamethasone (2.5 nmol/cm^2) and Triac (10 nmol/cm^2) (b), Triac (10 nmol/cm^2) (c). a and b show examples from Experiment 1; c is from Experiment 2 (see Table 1).

DISCUSSION

These experiments clearly demonstrate that Triac has an inhibitory effect on the skin atrophy induced by betamethasone in haired mice, as measured in histological sections using van Gieson staining. The reason we did not chose hairless animals as a model is that in the hairless rat, the hairless (hr) gene is mutated and the hr gene product interacts specifically with the thyroid hormone receptor and is regulated by thyroid hormone levels (13, 14). Our results are still comparable with those described in the hairless mouse with vitamin D_3 hormone analogues (11–12) and tretinoin (15).

Before accepting our results, the fact that we used haired animals as our model must be discussed because the stage of the hair cycle has an influence on the thickness of the skin (16). All the animals were of the same age, and by clipping the hair at the same time, the hair growth in all of the mice was placed in an anagen phase (16). In our experiments we used dose ranging of the active substance to distinguish between the pharmacological effects and those that could be related to a shaving effect. For example, if dermal thickness changes were due to shaving of the skin, they would have been observed at all concentrations of the drug used, both low and high concentrations. This was not observed, but a gradual increase in dermal thickness was observed as the concentration of the active substance changed from low to high.

Gniadecki et al. used a similar model system as the one used by us (11, 12). They reported the rapid onset of a 50% reduction of skin thickness within one week of bethamethasone administration at the same topical dosage as in our studies. This atrophy was rapidly and completely reversed within 2 to 4 weeks with the vitamin D analogue KH-1060 (11, 12). Although it is commonly assumed that collagen and glycosaminoglycan synthesis sufficient to double the skin thickness requires a much longer time-frame than that used in these experiments, the authors showed a marked increase in both proline and sulphate incorporation in the bethamethasone-KH1060 treated skin as compared with bethamethasone-treated skin. In the experiments reported here, no biochemical analysis was performed. However, our results occur in a time-frame similar to that reported by Gniadecki et al. (11, 12) and thus we speculate that the increased dermal thickness observed in our experiments could have resulted from these biochemical processes.

Triac was the most potent agent of the various TH analogues tested. Another interesting finding was that Triac given alone increased the dermal thickness compared to non-treated skin. In high concentrations, two other TH analogues, KB-026 and Triprop, also had an inhibitory effect on skin atrophy induced by topical corticosteroid treatment. However, Triac was the most effective compound and therefore the most interesting of the TH analogues to be further evaluated in clinical studies. Further studies are necessary to investigate the specific mechanism of action of TH analogues on the dermis.

Note added in proof:

During the review of this paper, a novel set of experiments disclosing the topical effects of triiodothyronine, an endogenous TH not included in our experiments, was published by Safer et al. (17). Using a shaved rat model they observed a similar increase in

dermal thickening (25% increase after 2 weeks) as we observed with Triac.

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