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SOFT DRUGS—10. BLANCHING ACTIVITY AND RECEPTOR BINDING AFFINITY OF A NEW TYPE OF GLUCOCORTICOID: LOTEPREDNOL ETABONATE

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Summary—An improved synthesis of loteprednol etabonate (chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene 17β-carboxylate) was achieved. The design of the new type of glucocorticoid was based on the soft drug concept. The relative binding affinities of loteprednol and its putative metabolites (PJ90 and PJ91) to rat lung type II glucocorticoid receptor were determined in a competitive binding experiment with [^{3}H]triamicinolone acetonide. The medium contained cortienic acid (10^{-5} M) in order to block transcortin binding sites. Loteprednol etabonate exhibited a binding affinity which was 4.3 times that of dexamethasone, both compounds having a Hill factor close to 1 whereas PJ90 and PJ91 did not show any affinity to the receptor. Loteprednol etabonate was compared to betamethasone 17α-valerate in a vasoconstriction test which was performed on the forearm skin of human volunteers. The results showed that loteprednol etabonate has good skin-permeation properties and strong glucocorticoid activity.

INTRODUCTION

Topical application of potent glucocorticoids, e.g. clobetasol 17-propionate, can produce severe toxic responses. Cushingoid features, pituitary–adrenal suppression [1], skin atrophy and inhibition of wound healing [2] have been observed. These types of actions result from the apparent ubiquitous and unique nature of the glucocorticoid receptor. Other kinds of toxic responses, i.e. allergies and cataract, which are observed in patients undergoing long-term glucocorticoid therapy, are believed to be related to the presence of an α-hydroxycarbonyl function [3]. In trying to design safer glucocorticoids, Bodor and co-workers [4–7] have addressed both aspects of the problem. They designed molecules that lack the reactive α-hydroxycarbonyl function and which are systemically non-toxic while being locally active. They based their approach on the newly-developed concept of soft drugs, in which toxicity considerations are integrated in the early stages of the drug design process. We describe here one of the most promising candidates, loteprednol etabonate, which has entered clinical trials in the U.S.A. In this first report, an improved synthesis of the new glucocorticoid is described. The new molecule is intrinsically potent, whereas the putative metabolites are intrinsically inactive.

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EXPERIMENTAL

Chemistry

Uncorrected melting points were recorded on a Fisher–Johns melting point apparatus. The proton NMR spectra were recorded on a varian EM390 spectrometer and the IR spectra were recorded on a Perkin Elmer 1600 FTIR. The elemental analysis was done by Atlantic Microlab (Atlanta, Ga). Optical rotations were measured on a Perkin–Elmer 141 polarimeter.

17α-Ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylic acid ethylcarbonic anhydride (4)

Compound **3** (50 g, 0.144 mol) was dissolved in water (500 ml) containing sodium bicarbonate (121 g). Methylene chloride (500 ml) was added, followed by tetrabutylammonium hydrogensulfate (285 g, 7.20 mmol). The reaction mixture was stirred vigorously and ethylchloroformate (47.9 g, 0.434 mol) was added, drop-

wise, over a period of 15 min. After stirring for 3 h, the organic phase was separated and was evaporated to dryness. The residue was dissolved in acetone (300 ml) and was precipitated by pouring the acetone in stirring cold water (2500 ml). The crystals were left in cold water for 16 h and were filtered. The product was dried *in vacuo*. The yield was 67.33 g (95%) of a white powder.

m.p.: 198.3–198.5°C.

H-NMR ($CDCl_3$): δ (ppm) = 1.13 (s, 18-CH_3); 1.27 (t, $J_{AX} = 7$ Hz, CH_2CH_3); 1.34 (t, $J_{AX} = 7$ Hz, CH_2CH_3); 1.44 (s, 19-CH_3); 4.13 (q, $J_{AX} = 7$ Hz, CH_2CH_3); 4.30 (q, $J_{AX} = 7$ Hz, CH_2CH_3); 4.47 (m, 11α-H); 5.98 (s, 4-CH); 6.23 (m, 2-CH); 7.27 (m, 1-CH).

IR(KBr): ν (cm^{-1}) = 3376, 1821, 1748, 1658.

Elemental analysis—theor: C, 63.66; H, 6.99.
found: C, 63.66; H, 7.01.

Chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene 17β-carboxylate (6)

Compound **5** (10 g, 0.024 mol) was dissolved in water (100 ml) containing sodium bicarbonate (9.90 g). Methylene chloride was added (80 ml), followed by tetrabutylammonium hydrogensulfate (0.47 g, 1.18 mmol). Chloromethylchlorosulfate (4.75 g, 0.029 mol) in methylene chloride (20 ml) was added dropwise over a period of 30 min while stirring vigorously. After stirring for 2 h, the organic phase was separated, dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give a crude yield 10.66 g. The product was crystallized from hot acetone (100 ml).

m.p.: 233–234°C.

$[\alpha]_{23}^{d} = 41.8 \pm 0.35$ ($C = 1.23 \pm 0.24$, CH_2Cl_2). The value was obtained from 6 independent measurements.

H-NMR ($CdCl_3$): δ (ppm) = 1.07 (s, 18-CH_3); 1.26 (t, $J_{AX} = 7$ Hz, CH_2CH_3); 1.44 (s, 19-CH_3); 4.11 (q, $J_{AX} = 7$ Hz, CH_2CH_3); 4.49 (m, 11α-H); 5.76 (ABq, CH_2Cl); 6.01 (s, 4-CH); 6.24 (m, 2-CH); 7.30 (m, 1-CH).

IR(KBr): ν(cm^{-1}) = 3346, 1772, 1742, 1654.

Elemental analysis—
theor: C, 61.73; H, 6.69; Cl, 7.59.
found: C, 61.82; H, 6.83; Cl, 7.66.

Binding assay

Unlabeled chemicals were obtained from Sigma Chemical Co. (St Louis, Mo.). 1,2,4-^{3}H-triamcinolone acetonide (45 Ci/mmol) was purchased from New England Nuclear (Boston, Mass). All the steps of the binding assay, up to the charcoal separation procedure, were performed in ice at 0°C.

Cytosol preparation. Male Sprague–Dawley rats, 150–200 g, were used 6 days after adrenalectomy. The rats were killed by decapitation. Immediately after resection of the lungs, the tissue was frozen in liquid nitrogen. The cytosol was prepared with a slight modification of a previously described method [8]. The frozen tissue was ground to small pieces. After addition of 6 vol of ice-cooled incubation buffer (10 mM Tris/HCl, 10 mM sodium molybdate, 2 mM 1,4-dithiothreitol), the tissue was homogenized with a Virtis 45 homogenizer, at full speed, for 4 periods of 45 s with a 1-min cooling period between each step. The homogenate was centrifuged at 105,000 *g* for 1 h at 4°C in a Beckman ultracentrifuge L8-70M equipped with a T170 rotor. Portions (5 ml) of the cytosol were frozen in liquid nitrogen and stored at −80°C.

Binding studies. The cytosol was thawed in a water bath and was diluted with 2–4 parts of incubation buffer containing a 15 mM concentration of diisopropyl fluorophosphate. To 160-μl portions of the diluted cytosol were added 20-μl portions of a stock solution of [^{3}H]triamcinolone acetonide in incubation buffer. The final concentration of tracer in each incubation medium was 10 nM. A solution of cortienic acid in buffer was also added to each sample so that the final concentration of cortienic acid in the incubation medium was 10^{-5} M. Finally, each sample received an identical volume of various concentrations of competitor (loteprednol) in ethanol. After a 24-h incubation period at 4°C, the unbound steroid was removed by addition of a 2% suspension of activated charcoal in buffer (400 μl). The mixture was incubated for 10 min in ice and then was centrifuged at 10,000 *g* for 3 min in a Fisher microcentrifuge. The radioactivity (cpm) in the supernatant (400 μl) was determined by liquid scintillation counting. The non-specific binding was determined in the presence of unlabeled dexamethasone (10^{-6} M) and was in all cases < 10% of the total binding. All determinations were performed in duplicate. The IC_{50} value of the investigated steroid (concentration of competitor necessary to displace 50% of the bound [^{3}H]triamcinolone acetonide) and the slope factor of the resulting competition curve were determined a non-linear curve fitting method using the NON-LIN module of the Macintosh

SYSTAT version. The data were fitted to the following logistic function:

$$B = T - T * C^N/(C^N + IC_{50}^N) + NS,$$

where

B = cpm in the presence of competitor,
T = cpm in the absence of competitor,
C = competitor concentration,
N = Hill slope factor

and

NS = cpm under non-specific binding conditions.

The resulting IC_{50} value was transformed into relative binding affinity (RBA) using dexamethasone (RBA = 100) as the reference standard:

$$RBA(x) = IC_{50}(\text{dexamethasone})/IC_{50}(x) * 100.$$

Vasoconstriction test

Solutions of loteprednol etabonate (0.01, 0.001 and 0.0001 M) and of betamethasone 17α-valerate (0.01 M), purchased from Sigma, were prepared in a mixture consisting of ethanol and isopropyl myristate in a ratio 90:10. The test solutions (0.05 ml) were applied to circular patches, 1-cm dia. The patches were in turn randomly applied to the previously cleansed and dried flexor surface of the forearms of 4 human volunteers. The application site was covered with a water-impervious film. The film was removed after 4 h and the intensity of the pallor was judged after 1, 2, 3, 4, 5 and 8 h by an experienced observer. The blanching was evaluated on a 0–4 scale, with half-units. The grading scale was as follows: 0 = normal skin; 1 = slight pallor of indistinct outline; 2 = pallor with at least two corners outlined; 3 = even pallor with a clear outline of the application site; 4 = very intense pallor. There were 11 patches for each different concentration of loteprednol and 9 patches for the control compound, betamethasone 17α-valerate.

RESULTS

Design

The approach used in the design of loteprednol etabonate (**6**) is known as the inactive metabolite approach, in which the first step (Fig. 1) is a retro-metabolic transformation of a putative *inactive* metabolite in order to identify potentially useful metabolic precursors. The second step is a recognition of the biologically *active precursors*. The recognition process is based on the available knowledge of structure–activity relationships (SAR). Potentially toxic structures are rejected at that stage. In the last step, the emerging candidates undergo minor structural modifications in order to tailor their biological activity to therapeutic needs. In Bodor's approach, the design of loteprednol relies on non-specific esterases for its deactivation. By analogy with cortienic acid (**1**), and endogenous inactive metabolite of hydrocortisone in man, it was postulated that **3** and **5** are biologically inactive entities. The synthesis of loteprednol therefore elaborates on **3**. Retro-metabolic transformation of **3** leads to **5**, an other inactive entity, whereas **6** is designed to be the immediate metabolic precursor of **5** and, by extension, of **3**.

Chemistry

The original synthetic method is described elsewhere [9]. The improved synthesis of **4** and

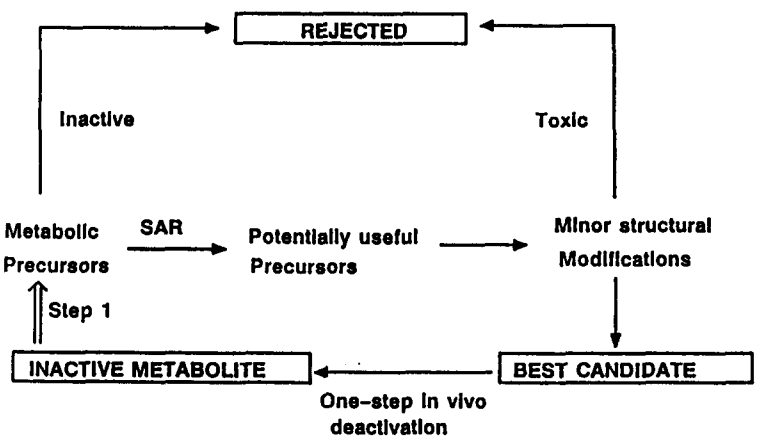
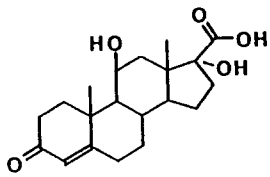


Fig. 1. Schematic view of the inactive metabolite approach.



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Scheme 1

6 is reported in the Experimental section. The synthesis of **6** proceeds according to the route described in Fig. 2. Prednisolone **2** was easily oxidized to the 17β-carboxylate **3** using an adaptation of the literature method for the oxidation of hydrocortisone [10]. The synthesis of the mixed anhydride **4** and **6** was greatly improved over the original method by the use of a liquid–liquid biphasic method and a transfer catalyst. In the last step, chloromethylchlorosulfate [11] showed a definite advantage over other alkylating agents, giving a product of higher purity and also a better yield.

Glucocorticoid receptor binding affinity

The relative affinities of the synthesized compounds to the glucocorticoid receptor were determined in a competitive binding experiment using rat lung cytosol as a standard receptor source [12]. A first experiment performed in the absence of cortienic acid revealed a Hill coefficient of 1.32–1.51 for loteprednol, while dexamethasone exhibited a coefficient close to 1.0 as expected (data not shown). In the presence of cortienic acid, at a concentration

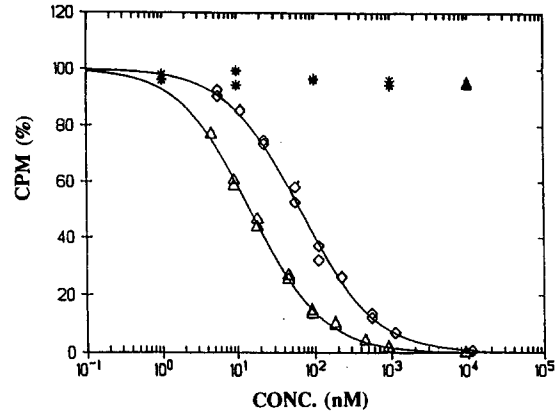

Fig. 3. Competition curves of loteprednol etabonate (△), dexamethasone (◇), PJ-91 (*) and PJ-90 (▲) in the presence of cortienic acid.

sufficient to block the transcortin binding sites [13], the Hill coefficient of loteprednol was close to 1.0. This observation indicates a possible binding affinity of loteprednol for transcortin, which would be predicted for a non-fluorinated glucocorticoid [14]. The affinity of loteprednol for the rat lung glucocorticoid receptor is 4.3 times that of dexamethasone (Fig. 3), whereas the potential metabolites PJ90 and PJ91 do not bind significantly. This finding agrees with the previously observed lack of affinity exhibited by glucocorticoids with a 17β-carboxylic function. This observed affinity of **6** is substantially higher than expected from an analog of **2**. While it is not possible to draw any conclusion from this simple comparison, other data seem to relate this increase in binding activity to the presence of a lipophilic substituent at the

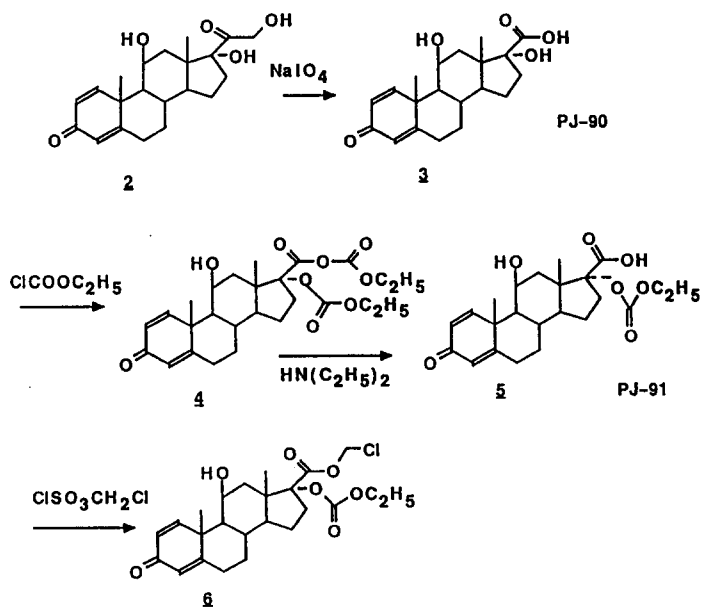

Fig. 2. Synthetic scheme of loteprednol etabonate.

17α-position [15] as well as to the presence of a chlorine atom at a position similar to that of the potent 21-chlorinated glucocorticoids [4]. There are literature data on the binding affinities of commercially relevant glucocorticoids to the receptor of rat and human lungs [16–18]. Since it appears that there is no significant difference between the binding properties of the glucocorticoid receptor in rat and human tissues [16], a first approximation places the intrinsic activity of loteprednol somewhere between that of triamcinolone acetonide (RBA = 233 [16]) and budesonide (RBA = 780 [18]), two potent glucocorticoids.

Vasoconstriction test

A human vasoconstriction test was performed in order to confirm the glucocorticoid nature of **6**. This test was originally designed to assess the percutaneous absorption of glucocorticoids [19]. It was later modified and extented so that it may be used to screen for activity and to determine bioavailability of topical steroids [20]. In this study the data were expressed in terms of index of activity, as already described by Barry and Woodford [20]. The results (Fig. 4) clearly show a concentration-dependency, as well as a time-dependency, of the response curve, as expected from our present knowledge of the kinetics of skin permeation. The topical application of **6** produced a blanching response which was similar to that of betamethasone 17α-valerate, a potent topical glucocorticoid. Betamethasone 17α-valerate has a binding affinity, in cultured human skin fibroblasts, which is in the range of that of **6** [21]. Since the degree of pallor depends both on the intrinsic potency of the agonist at the receptor and on the amount of compound reaching its site of action, it may be concluded that **6** is a potent glucocorticoid with good permeation properties.

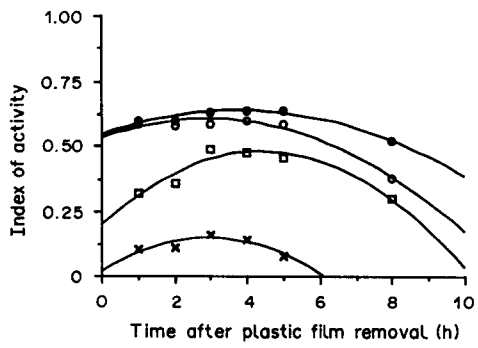

Fig. 4. Vasoconstriction activity of loteprednol etabonate 0.01 M (○), 0.001 M (□) and 0.0001 M (×), and of betamethasone 17α-valerate 0.01 M (●).

CONCLUSION

A new type of glucocorticoid was synthesized. The design of the new molecule was based on the soft drug concept. This study successfully demonstrated that the starting inactive putative metabolites are indeed intrinsically inactive, in accord with the requirements for the soft drug design, whereas loteprednol itself is intrinsically 4.3 times more potent than dexamethasone. Soft drugs are designed to be locally active, and as such they are especially useful for topical application. Loteprednol possesses a metabolically labile function, the 17β-ester, that is designed to be rapidly deactivated in the systemic circulation. After exerting its therapeutic activity at the desired site, the soft drug is expected to be rapidly biotransformed to generate the starting inactive metabolites PJ-90 and PJ-91, which in turn should be rapidly eliminated. Consequently, the compound is expected to have local antiinflammatory properties while being systemically innocuous.

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