Metabolism, Distribution, and Transdermal Permeation of a Soft Corticosteroid, Loteprednol Etabonate

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The soft corticosteroid, loteprednol etabonate (chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate), **I**, was designed based on the "inactive metabolite approach." Accordingly, **I** should be metabolized by hydrolysis to the corresponding inactive cortienic acid derivative, **II**. The *in vitro* and *in vivo* metabolism of **I** indeed yielded mainly this inactive metabolite, which is more hydrophilic and thus readily eliminated from the body. Relatively high levels of **I** were found in tissues after intravenous administration of the drug in rats. The permeability of **I** through hairless mouse skin was comparable to what has been found for related "hard" steroids, without significant metabolism taking place in the skin.

KEY WORDS: soft corticosteroid; loteprednol etabonate; metabolism; tissue distribution; transdermal permeability.

INTRODUCTION

In general, the main objective of drug design is to improve the therapeutic activity, i.e., design, of more potent drugs. Unfortunately, this increase in drug potency frequently leads to a parallel increase in toxicity, particularly for drugs that have multiple activities, such as corticosteroids. Drug toxicity can result from pharmacological effects of the drug itself, but also from effects of the drug metabolites or intermediates. The concept of soft drugs was put forward to provide specific methods for introducing structure–metabolic relationships into the drug design process (1–7). This approach to drug design is aimed at controlling and directing metabolism by design. Soft drugs can be defined as biologically active compounds (drugs) characterized by a predictable and controllable active destruction (metabolism) to nontoxic products (metabolites) after they have achieved their therapeutic effect (7). Ideally, the soft drug is inactivated in one metabolic step.

There are at least five approaches to soft drug design: soft analogue, activated soft compounds, natural soft drugs, soft drugs based on the activated metabolic approach, and those based on the inactive metabolite approach (1,2,7). The soft corticoid was designed by the inactive metabolite approach, which can be summarized as follows.

1. A pharmacologically inactive and nontoxic metabolite of a specific drug is identified and used as the lead compound.
2. Chemical modifications are performed on this metabolite to obtain structures that resemble (isosteric and/or isoelectronic) that of the drug from which the lead inactive metabolite was derived. This is the activation step.
3. The structure of the new soft drug is designed in such a way that its metabolism will yield the original inactive metabolite without going through toxic intermediates.
4. The transport and binding properties, as well as the rate of metabolism and the pharmacokinetics, are controlled by molecular manipulations in the activation stage.

According to this approach, several soft corticosteroids have been synthesized and tested. One of the most promising soft corticosteroids tested so far is loteprednol etabonate, **I**, which is currently undergoing clinical trials in humans. In this case the lead compound was the inactive metabolite of prednisolone, Δ^1-cortienic acid, **III**. The synthesis has been described elsewhere (8,9). The structures of **I** and its proposed metabolites, **II** and **III**, are shown in Fig. 1.

A soft steroid such as **I** is intended to be used only topically, including skin, lung, colon, eye, etc., as its facile systemic metabolism (by design) would make its systemic use meaningless. Accordingly, oral administration of **I**, even at high doses, resulted in minimal thymolytic and adrenal suppressive effects (10).

The topical antiinflammatory activities of **I** have been shown to be similar to that of betamethasone, which is one of the most active antiinflammatory steroids (8). However, **I** causes far fewer side effects even locally than the traditional corticosteroids (8,11). This study describes the initial investigation of the metabolism and distribution of **I** in rats, as well as the permeability of **I** through hairless mouse skin.

MATERIALS AND METHODS

Materials

Loteprednol etabonate, **I**, was supplied by the courtesy of Otsuka Pharmaceutical Co. (Japan). Δ^1-Cortienic acid etabonate, **II**, was supplied by the courtesy of Xenon Vision Inc. (Alachua, FL). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DMCD) was obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals were commercially available products of special reagent grade.

Male Sprague Dawley rats weighting 200 to 250 g were obtained from Charles Rivers (Wilmington, MA) and female hairless mice, 12 to 14 weeks old, from Temple University (Philadelphia, PA).

EXPERIMENTAL METHODS

In Vitro Transformation in Rat Blood and Plasma

Freshly obtained rat trunk blood and plasma were used. Animals were sacrificed by decapitation. Fifty microliters of **I** in acetonitrile was added to 5 ml of whole blood or plasma

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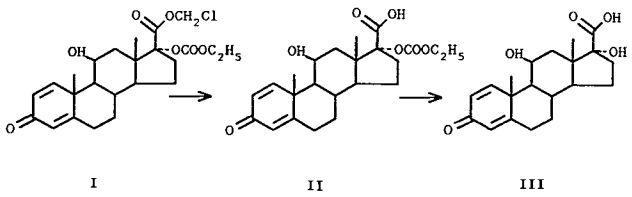

I II III

Fig. 1. Loteprednol etabonate and its proposed metabolites.

at 37°C, giving a final concentration of 30 μg/ml. At appropriate time intervals, a 0.1-ml sample was taken and mixed with 0.2 ml of 5% dimethyl sulfoxide (DMSO) in acetonitrile solution. After centrifugation, the supernatant was diluted 1:1 with mobile phase and injected in HPLC.

In Vivo Metabolism in Rats

Male Sprague–Dawley rats were anesthetized by intraperitoneal injections of pentobarbital (30 mg/kg). Aqueous 20% (w/w) heptakis(2,6-di-*O*-methyl)-β-cyclodextrin solution containing 5 mg/ml of I was injected in the femoral vein at a dose of 20 mg/kg. At appropriate time intervals, 0.1 ml of blood was taken from the jugular vein, mixed with 0.2 ml of 5% DMSO in acetonitrile solution and centrifuged, and the supernatant was analyzed by HPLC. The metabolite of loteprednol etabonate, II, 4 mg/ml in 20% (w/w) heptakis(2,6-di-*O*-methyl)-β-cyclodextrin solution, was injected at a dose of 10 mg/kg and the samples were taken and treated by the same procedure as described before.

Data obtained from intravenous injection of I or II were fit in a three-compartment model using a nonlinear regression program RSTRIP.

In Vivo Distribution in Rats

Male Sprague–Dawley rats were anesthetized and injected with aqueous 20% (w/w) heptakis(2,6-di-*O*-methyl)-β-cyclodextrin solution containing 5 mg/ml of I as before. At various time points the animals were sacrificed by decapitation, and samples of blood, brain, lung, kidney, and liver were collected. The samples were weighed and mixed or homogenized immediately with two times their weight of 5% DMSO in acetonitrile solution. The homogenate was centrifuged and the supernatant was analyzed by HPLC.

Skin Permeability Studies

Female hairless mice were sacrificed by cervical dislocation. The whole dorsal skin was removed, placed carefully over a circular Teflon holder, and held in place with an O-ring. This yielded a 7.07-cm^2 skin surface which was suspended over a plexiglass reservoir (diffusion cells from Kercso Engineering, Palo Alto, CA) containing 40 ml of receptor phase. The receptor phase consisted of pH 7.4 isotonic phosphate buffer containing 0.3% Brij-58 (polyoxyethylene 20 cetyl ether), which was sonicated under vacuum prior to usage to remove the dissolved air. An excess of I was added to the vehicle (donor phase) and the suspension formed was sonicated for 2 hr. After equilibration at 34°C for 12 hr the suspension was spread over the skin surface (about 3 ml on each skin). The cells were placed on magnetic stirrers in a 34.0 ± 0.5°C incubator, which contained a pan of water for humidity control, and kept there under gentle stirring for 48 hr. Samples of receptor were removed from the cells at various time intervals and replaced with fresh buffer solution. The samples were subsequently kept frozen until analyzed by HPLC.

The solubility of I in the vehicles was determined by adding an excess amount of I to a vehicle and sonicating the mixture for 1 hr. After equilibration in a 34°C incubator for 5 hr, an aliquot of the mixture was filtered through a 0.45-μm membrane filter, diluted, and analyzed by HPLC. The steady-state flux was obtained from the linear portion of individual cumulative amounts versus time plots. The permeability coefficient was calculated by dividing the flux by the solubility of I in the donor phase (12). Each experiment was repeated at least three times, and the results reported are the mean values.

Analytical Method

A high-performance liquid chromatographic (HPLC) method was developed for the quantitative determination of I and II. A NOVA-PAK phenyl column (4 μm, 3.9 × 7.5 cm) from Waters was connected to a Spectra-Physics component system consisting of a SP 8810 isocratic pump, Rheodyne 7125 injector (10-μl injection volume), SP 8450 UV/Vis detector (operated at 254 nm), and SP 4290 integrator. The system was operated at ambient temperature. The mobile phase consisted of acetonitrile, acetic acid, and water (45:1:54). At a flow rate of 1.5 ml/min, the retention time of I was 4.2 min and that of II was 1.3 min. The concentration of the compounds was determined by comparing the peak area with the corresponding standard peaks. The extraction ratio of I and II was, on the average, 99.6 ± 5.5 and 101.0 ± 4.0%, respectively.

RESULTS AND DISCUSSION

In Vitro Transformation in Rat Blood and Plasma

Soft drug, I, possesses a metabolically labile function group, the 17-β-ester, which is designed to be rapidly deac-

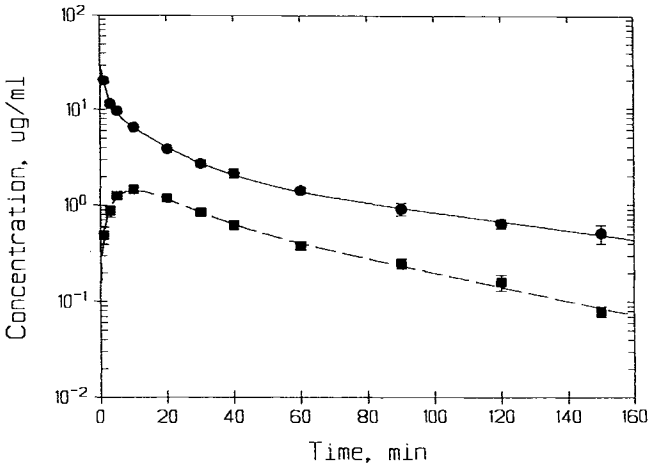

Fig. 2. Mean blood concentration–time profiles of loteprednol etabonate, I (●), and its metabolite, II (■), following intravenous administration of 20 mg/kg of I in rats.

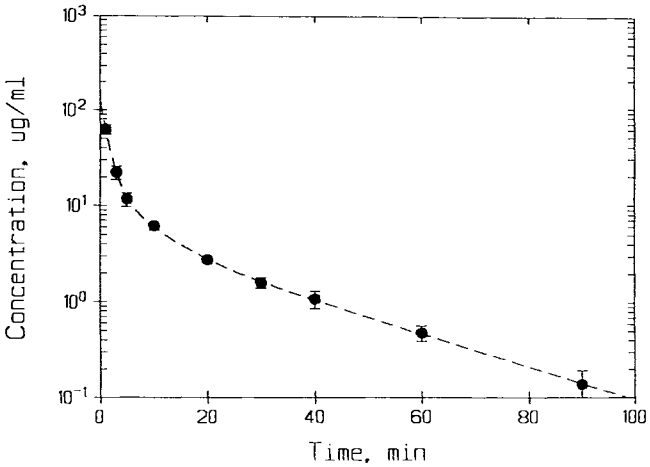

Fig. 3. Mean blood concentration–time profile of Δ^1-cortienic acid etabonate, II, following intravenous administration of 10 mg/kg of II in rats.

tivated systemically, forming the expected inactive metabolite, II; during *in vitro* studies in rat blood or plasma, the biotransformation of I was found to be relatively fast. The degradation half-lives in blood and plasma were 8.91 ± 0.48 min (r = 0.998, three experiments) and 4.89 min (r = 0.998, one experiment), respectively. The rate of formation of II was approximately equal to the disappearance of I, which indicates that the designed metabolite, II, was the main degradation product.

In Vivo Metabolism in Rats

Figure 2 shows the mean blood levels of I and II following intravenous administration of 20 mg/kg of I in rats. From the linear portion of the curves, the half-lives of I and II in the terminal phase were determined to be 51.4 ± 1.42 and 39.5 ± 2.69 min, respectively. Also, in Fig. 3, after 10 mg/kg of II was administered in rats, the terminal-phase half-life of II was determined to be 20.42 ± 1.30 min, which is much shorter than after administration of I, since in that case II is still forming, which makes the slope less negative. The total-body clearance of the metabolite II was found to be about four times greater than that of I (~83 vs ~24 ml/min/kg). These results indicate that *in vivo* in rats, the metabolism of I indeed follows the predicted degradation pathway, and the inactive metabolite, II, is rapidly eliminated from the body.

In Vivo Distribution in Rats

The anesthetized animals were sacrificed at 5, 40, and 150 min after intravenous administration of 20 mg/kg of I. The concentrations of I and II in blood, brain, kidney, liver, and lung were determined (Table I). The distribution of I was consistent with the lipophilic nature of the molecule. The compound penetrated the blood–brain barrier easily and was widely distributed in other tissues. The concentration of I in tissues tested was, in general, much higher than in blood ($P < 0.05$). The high concentrations of both I and its metabolite, II, in the liver indicate that this organ is the main site of metabolism when I is given systemically. Urinary excretion of I and II from 0 to 150 min indicated a total amount of I and II of less than 5% of the dose administered. The high initial concentration of I in the kidney is probably due to the high dose in this preliminary investigation. Some of the metabolite II is further hydrolyzed to III, but concentrations of III were not determined here: generally, they are much lower than that of II. Detailed metabolism studies of I *in vivo* in rabbit eye (13) have also shown that II is the main metabolite, and its concentration in some eye compartments, such as the cornea and aqueous humor, exceeds that of I. In addition, recent unpublished studies indicate that significant amounts of the metabolite II are eliminated via the bile. Thus, the actual plasma concentrations of II after intravenous administration of I do not reflect the extent of conversion of I to II. The present studies, however, clearly demonstrate that II is the main metabolite of I.

Skin Permeability *in Vitro*

The effect of various vehicle systems on the transdermal

Table I. The Concentration of Loteprednol Etabonate, I, and Δ^1-Cortienic Acid Etabonate, II, in Blood and Various Tissues After Intravenous Administration of 20 mg/kg of I to Rats[a]

Time (min)	Blood	Brain	Kidney	Liver	Lung
			Concentration of I (μg/ml or μg/g)		
5	2.26 ± 3.6	35.2 ± 3.4*	99.5 ± 28.1*	102.0 ± 7.1*	41.8 ± 6.1*
40	2.6 ± 0.2	8.2 ± 0.7*	72.8 ± 9.7*	15.3 ± 2.4*	8.8 ± 0.6*
150	0.51 ± 0.14	—[b]	0.43 ± 0.10	1.1 ± 0.1*	—
			Concentration of II (μg/ml or μg/g)		
5	1.8 ± 0.4	0.32 ± 0.03	5.1 ± 0.25	9.1 ± 2.2*	2.3 ± 0.3
40	1.9 ± 0.2	1.3 ± 0.2	11.0 ± 1.5*	10.0 ± 0.5*	2.9 ± 0.6
150	0.10 ± 0.04	—	1.1 ± 1.0	0.07 ± 0.04	—

[a] The data reported are the mean value ± standard error of four animals.
[b] Not determined.
* Significant difference, $P < 0.05$.

Table II. The Vehicle Composition, the Solubility (C_d) of Loteprednol Etabonate, I, in the Vehicle, and the Permeability Coefficient (P) of I Through Hairless Mouse Skin[a]

Vehicle[b]	C_d (mg/ml)	P (cm/hr)
Water	0.001	—[c]
2% (v/v) OA in PG	2.45	$1.06 \pm 0.06 \times 10^{-5}$
2% (w/v) CL in PG	2.66	$9.92 \pm 0.44 \times 10^{-5}$
20% (w/v) DMCD in water	6.25	$4.08 \pm 0.72 \times 10^{-5}$
20% (w/v) DMCD, 1% (v/v) PG in water	7.76	$8.49 \pm 3.04 \times 10^{-6}$
40% (w/v) DMCD in water	17.8	$5.4 \pm 0.17 \times 10^{-5}$
40% (w/v) DMCD, 1% (v/v) PG in water	18.0	$5.65 \pm 0.61 \times 10^{-5}$
10% (w/v) DMCD, 10% (v/v) PG in water	1.41	$1.03 \pm 0.15 \times 10^{-5}$

[a] Each experiment was repeated three to five times and the values shown are the mean values ± standard error.

[b] PG, propylene glycol; OA, oleic acid; CL, choline laurate; DMCD, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin.

[c] No loteprednol etabonate or Δ^1-cortienic acid etabonate was detected on the receptor side.

delivery of I was investigated. The permeability coefficient, which is an index of how well the drug molecule permeates the skin, was used for the comparison. As shown in Table II, the permeability coefficients obtained ranged from 1×10^{-4} to 1×10^{-5} cm/hr, which shows that the soft corticosteroid, I, permeates the skin at a rate which is comparable to what has previously been found for other nonsoft steroids (14). In this *in vitro* study, about 5 to 7% of II was detected in the receptor phase after the permeation process, which was estimated from the I/II concentration ratios formed in the receptor phase and the rate constant for the degradation of I and II in the solution (1.7×10^{-2} min^{-1}).

CONCLUSIONS

The soft corticosteroid, loteprednol etabonate, I, possesses antiinflammatory activity which is comparable to that of betamethasone, with fewer side effects (8,10). This fact by itself demonstrates the soft character of the compound, which can be explained by the simplified inactivation process *in vivo* as we have reported in this study. I is hydrolyzed mainly to the predicted inactive metabolite, II. This metabolite is more hydrophilic than loteprednol and is eliminated from the body directly or, possibly, in conjugated forms. The permeability of the lipophilic soft corticosteroid I through biological membranes is good, resulting in relatively high concentrations of the compound in brain and other organs after intravenous administration and efficient transdermal delivery after topical application. This study demonstrates a successful design of a soft corticosteroid which currently is undergoing phase 2 clinical trials.

REFERENCES

1. N. Bodor, J. Kaminski, and S. Selk. Soft drugs 1. Labile quaternary ammonium salts as soft antimicrobials. *J. Med. Chem.* 23:469–474 (1980).
2. N. Bodor, R. Wood, C. Raper, P. Kearney, and J. J. Kaminski. Soft drugs 3. A new class of anticholinergic agents. *J. Med. Chem.* 23:474–480 (1980).
3. N. Bodor. Designing safer drugs based on the soft drug approach. *Trends Pharmacol. Sci.* 3:53–56 (1982).
4. N. Bodor. Soft drug: Strategies for design of safer drugs. In K. Buisman (ed.), *Strategy for Drug Design,* Elsevier Scientific, Amsterdam, 1982, pp. 137–164.
5. N. Bodor, Y. Oshiro, T. Loftsson, M. Katovich, and W. Caldwell. Soft drugs VI. The application of the inactive metabolite approach for design of soft β-blockers. *Pharm. Res.* 3:120–125 (1984).
6. N. Bodor and T. Loftsson. Novel chemical approaches for sustained drug delivery. In J. R. Robinson and V. H. L. Lee (eds.), *Controlled Drug Delivery: Fundamentals and Applications,* Marcel Dekker, New York, 1987, pp. 337–371.
7. N. Bodor. The application of soft drug approaches to the design of safer corticosteroids. In E. Christophers *et al.* (eds.), *Topical Corticosteroid Therapy: A Novel Approach to Safer Drugs,* Raven Press, New York, 1988, pp. 13–25.
8. N. Bodor. Novel approaches to the design of safer drugs: Soft drugs and site-specific chemical delivery systems. In *Advances in Drug Research, 13,* Academic Press, London, 1984, pp. 255–331.
9. P. Druzgala, G. Hochhause, and N. Bodor. Soft drugs. 10. Blanching activity and receptor binding affinity of a new type of glucocorticoid: Loteprednol etabonate. *J. Steroid Biochem.* 38(2):149–154 (1991).
10. J. Howes. Xenon Vision Inc. internal reports (1989).
11. N. Bodor and M. Varga. Effect of a novel soft steroid on the wound healing of rabbit cornea. *Exp. Eye. Res.* 50:183–187 (1990).
12. T. Loftsson. Experimental and theoretical model for studying simultaneous transport and metabolism of drugs in excised skin. *Arch. Pharm. Chem. Sci.* 10:17–24 (1982).
13. P. Druzgala, W. M. Wu, and N. Bodor. Ocular absorption and distribution of loteprednol etabonate, a soft steroid, in rabbits eyes. *Curr. Eye. Res.* 10:933–937 (1991).
14. T. Loftsson, B. J. Olafsdottir, and N. Bodor. The effects of cyclodextrins on transdermal delivery of drugs. *Eur. J. Pharm. Biopharm.* 37:30–33 (1991).