Soft Drugs 19. Pharmacokinetics, Metabolism and Excretion of a Novel Soft Corticosteroid, Loteprednol Etabonate, in Rats

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Purpose. Pharmacokinetics, metabolism and excretion of loteprednol etabonate (LE) were investigated in rats. ***Methods.*** The pharmacokinetic studies were performed by iv injections of LE (1-20 mg/kg). In the metabolism and excretion studies, 0.5-10 mg/kg of LE were iv administered, bile and urine samples were collected for 6 hr. ***Results.*** The pharmacokinetic of LE showed a rapid, dose-dependent elimination with a total blood clearance (CL_{total}) of higher than 60 ml/min/kg. The metabolism and excretion of LE also showed a marked dose-dependency. At 6 hr after iv of LE (0.5-10 mg/kg), the total recoveries (LE and the metabolites, AE & A, in bile and urine) were 99.35-26.72%. However, only about 2% of LE was excreted from the body through the urine. There were 0.93-2.12% and 0.66-0.26% of AE, and 75.67-19.69% and 20.74-2.77% of A excreted in the bile and urine, respectively. The excretion of A was dose dependent, and significantly higher at the lower dose. Using the (% of total excretion) vs. (log dose) plots, it could be predicted that almost all of the administered LE will be metabolized, and excreted as A when the systemic dose is lower than 0.25 mg/kg. ***Conclusions.*** The results indicate that LE absorbed systemically, after topical administration, can be rapidly transformed to the inactive metabolites, and eliminated from the body mainly through the bile and urine.

KEY WORDS: soft corticosteroid; loteprednol etabonate; pharmacokinetics; metabolism; excretion.

INTRODUCTION

Corticosteroids exhibit extremely potent anti-inflammatory properties when applied locally. However, prolonged therapeutic use of corticosteroids is frequently associated with a variety of undesirable systemic, toxic complications(1-2). These side effects can be very serious when the drugs were applied on irritated, inflamed mucous membrane where the permeability of the drugs increases (3). To improve the therapeutic index of corticosteroids, "soft drug" concept was introduced. This concept provides specific methods for introducing structure-metabolic relationships into the drug design process, thus the metabolism of a drug can be controlled (4-10). A soft steroid, loteprednol etabonate (LE), was designed, and its topical anti-inflammatory activity was tested and reported previously (10-12). LE was designed so that it undergoes a two-step metabolism *in vivo* first to the Δ^1-cortienic acid etabonate (AE), and then to the lead compound, Δ^1-cortienic acid (A), both inactive, in the body (10).

To assess the "controlled metabolism" of LE, the pharmacokinetics, metabolism and excretion of LE after intravenous administration were investigated in rats.

MATERIALS AND METHODS

Materials and Animals

Compounds, chemicals, as well as animals described previously (13) were used.

Intravenous Administration of LE

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). The opening of urethra was closed with a drop of surgical cement to prevent leakage of urine, and the jugular vein was exposed. LE was dissolved in a 50% hydroxypropyl-β-cyclodextrin (HPCD) aqueous solution and injected into the tail vein, over one minute, at a dose of 1, 2, 5, 10, 20 mg/kg and a dosing volume of 8 ml/kg. For the data treatment (as a bolus injection), the mid-time of the injection was used as 0 time. The tail vein injections were conducted very carefully to assure that no leakage occurred during the injections. Blood samples, 0.1 ml, were collected through the jugular vein at appropriate time intervals for 150 min. Subsequently, the urine samples were taken and animals were sacrificed by over dose of pentobarbital. The blood and urine samples, 0.1 ml, were added with 0.2 ml of acetonitrile containing 5% dimethylsulfoxide solution, mixed in the vortex mixer, and centrifuged at 3000 rpm for 5 min. The supernatant was then analyzed by HPLC. The recovery of both LE and AE were determined as 100 ± 3%.

Pharmacokinetics

Noncompartmental and compartmental pharmacokinetic analysis were performed. For noncompartmental analysis, the area under the curve, AUC, of the blood concentration versus time were calculated using the trapezoidal rule, and the area from the last measurement, C_t, to infinity was calculated as C_t/β, where β is the terminal disposition rate constant. The total body clearance, CL_{tot}, was calculated as Dose/AUC. Mean resident time, MRT, was calculated as AMUC/AUC, where AMUC, the area under the first moment curve, was calculated using the trapezoidal rule from the curve of blood concentration · time - time, and the area from the last time point, t, to infinity was calculated as $t \cdot C_t/\beta + C_t/\beta^2$. The volume of distribution at steady state, Vd_{ss}, was calculated as CL_{tot} multiplied by MRT.

For compartmental analysis, a curve-stripping program RSTRIP (Micro math, Salt lake city, UT) was used to assist the pharmacokinetic analysis. The results were best fitted in a two-compartment model, $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the drug concentration in blood, A and B are the exponential

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multipliers, α and β are the hybrid constants in the compartment 1 and 2, respectively. The AUC was calculated as $A/\alpha + B/\beta$, and the half-life of the terminal phase, $t_{1/2}$, was calculated as $\ln 2/\beta$. The volume of distribution of the central compartment, Vd_c, was calculated as Dose/A+B; the volume of distribution during the elimination phase, Vd_{area}, was calculated as CL_{tot}/β; and the elimination rate constant, K_{el}, was calculated as CL_{tot}/Vd_c.

Metabolism and Excretion of LE After Intravenous Administration

Animals were anesthetized as before. The peritoneal cavity was exposed and the common bile duct was cannulated using polyethylene tubing (PE 10, Clay Adams). LE solution was administered intravenously, as described above, at a dose of 0.5, 1, 2, 5 or 10 mg/kg. At varying time intervals (2, 4 and 6 hr), total bile juice was collected and weighed. After 6 hr, the urine samples were collected subsequently by a syringe, and animals were sacrificed with a lethal overdose of Na pentobarbital. For the detection of LE in bile and urine, samples were prepared as described above for the blood samples. For the analysis of AE and A, the bile and urine samples (0.1 ml) were mixed with 0.1 ml of 0.1 M HCl and 1 ml of ethyl acetate. The mixture was shaken by vortex mixer for 30 min, centrifuged at 3000 rpm for 5 min, and 0.8 ml of the upper organic layer was separated. The organic solvent was evaporated under nitrogen gas, and the residue was reconsitituted by 0.3 ml of 30% acetonitrile solution. The mixture was vortexed for 30 min, and injected in the HPLC system.

Analytical Methods

A HPLC method described previously (13) was used to analyze the concentration of LE, AE and A in the samples. A Waters Nova-Pak Radial-Pak cartridge (4 μm, 5 mm × 10 cm) was used in this study. A mobile phase consisted of acetonitrile, acetic acid and water in a volume ratio of 60: 0.2:40 was used for separating LE and AE in the blood samples as well as LE in the bile samples. At a flow rate of 1 ml/min, the retention time of LE and AE were 7.50 and 4.30 min, respectively. For the separation of AE and A in bile, a mobile phase consisted of acetonitrile, acetic acid and water in a volume ratio of 35:0.1:65 was used. The retention time of A and AE, at a flow rate of 1 ml/min, were 18.65 and 5.85 min, respectively. The detection limit was less than 0.1 μg/ml for all three compounds. Standard curves (0-1, 0-10 & 0-100 μg/ml) of each compound were developed by adding known amounts of the compound in the blank blood or bile samples, then prepared as described previously. The concentrations of the compound in the unknown samples were calculated by comparing the peak area with that of the corresponding calibration curves ($r > 0.990$).

RESULTS AND DISCUSSION

Pharmacokinetics in Rats After Intravenous Administration of LE

After intravenous bolus administration of various doses (1-20 mg/kg) of LE in rats, blood concentration-time curves were developed for the pharmacokinetic evaluations. As shown in figure 1, LE was eliminated from the blood in a bi-phasic manner, and marked dose-dependency was observed, i.e., the elimination rate of LE was greatly reduced at higher doses.

The data shown in figure 1 were analyzed by noncompartmental and compartmental method. The resulting pharmacokinetic parameters are listed in table 1. The concentration-time curves were very well described by an iv bolus two-compartment model according to a bi-exponential equation, $C = Ae^{-\alpha t} + Be^{-\beta t}$. The statistics on the correlation coefficient of variation, >0.995, and the model selection criterion, ranged 3.3-4.6, indicate the goodness of fit.

As the table displays, at the dose range of 1-20 mg/kg, the half lives, $t_{1/2}$, of LE in rats were fairly short. The CL_{total} value of LE was relatively high and displayed a dose dependent manner. However, at the highest dose examined (20 mg/kg), the CL_{total} value of LE, 60.4 ml/min/kg, was still higher than the physiological hepatic blood flow, 58 ml/min/kg in rat (14), indicating that LE transferred to the liver by the hepatic blood flow is completely extracted. This high CL_{total} value of LE, especially at the dose of lower than 5 mg/kg, also suggests that in addition to the metabolism in the liver, the enzymatic degradation in the blood or other elimination processes such as lung, intestinal or kidney metabolic clearances are involved in the clearance of LE from the systemic circulation (15). The volume of distribution of the central compartment, Vd_c, are not affected by the change of dose. However, the elimination rate constants, k_{el}, were much higher at lower dose (1 or 2 mg/kg) as in the case of CL_{tot}, indicating the capacity of the organism to eliminate LE at lower doses are higher.

Generally, ester-type agent is considered being easily and rapidly hydrolyzed by pseudo cholinesterase in the systemic circulation (16-17). In fact, the enzymatic degradation of LE into AE in blood has already been observed (18). In the clinical use of corticosteroid for the local treatment, such as GI, eye or skin inflammations, the normal dose range used topically is about 1-2 mg/kg or less (1,19,20). At this dosing

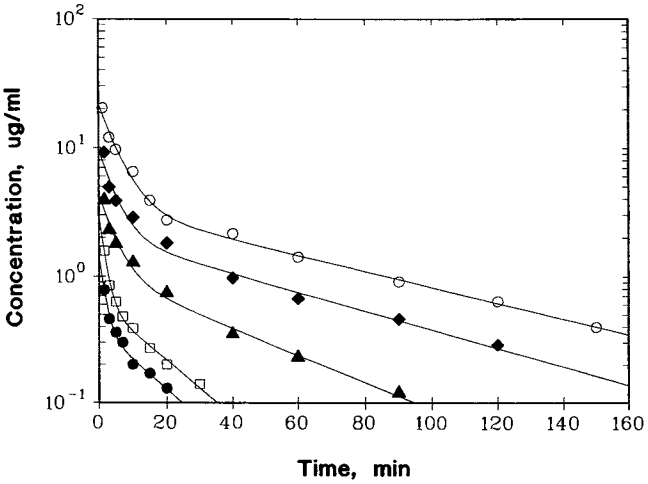

Fig. 1. Blood concentration - time profiles of LE in rats (n = 3) after intravenous bolus administration at various doses. 1 (●), 2 (□), 5 (▲), 10 (♦), 20 (○) mg/kg. Solid lines represent the two-compartment model fitting curves.

Table 1. Pharmacokinetics of LE after Intravenous Bolus Administration in Rats[a]

Dose, mg/kg	1	2	5	10	20
AUC, μg · min/ml[b]	9.2 ± 0.4	16.0 ± 1.1	56.1 ± 6.2	159.2 ± 31.3	333.2 ± 17.9
CL_{tot}, ml/min/kg[b]	108.5 ± 4.5	125.8 ± 9.0	90.3 ± 10.0	67.4 ± 11.6	60.4 ± 3.1
MRT, min[b]	17.95 ± 0.95	18.34 ± 0.80	31.98 ± 0.78	48.72 ± 8.95	51.79 ± 1.70
Vd_{SS}, ml/kg[b]	1952 ± 183	2312 ± 265	2894 ± 389	3078 ± 79	3121 ± 158
A, μg/ml	1.19 ± 0.46	1.69 ± 0.44	3.11 ± 0.38	8.65 ± 2.75	17.87 ± 0.71
B, μg/ml	0.32 ± 0.06	0.38 ± 0.15	0.51 ± 0.37	1.92 ± 0.25	3.41 ± 0.40
α, l/mln[c]	0.56 ± 0.27	0.47 ± 0.12	0.64 ± 0.36	0.28 ± 0.15	0.19 ± 0.01
β, l/min[c]	0.044 ± 0.003	0.041 ± 0.004	0.024 ± 0.000	0.017 ± 0.003	0.014 ± 0.000
$t_{1/2}$ (β), min[c]	15.92 ± 1.23	17.22 ± 1.71	29.49 ± 0.00	43.41 ± 7.58	48.82 ± 1.52
Vd_c, ml/kg[c]	749 ± 257	1004 ± 258	1444 ± 298	1092 ± 264	944 ± 45
Vd_{area}, ml/kg[c]	2249 ± 294	3145 ± 533	3842 ± 425	3987 ± 289	4253 ± 313
K_{el}, l/min[c]	0.162 ± 0.049	0.132 ± 0.025	0.067 ± 0.021	0.071 ± 0.024	0.064 ± 0.002
r[d]	0.995	0.998	0.996	0.998	0.995

[a] Each value represents Mean ± S.E. of three trials.
[b] Parameters obtained by noncompartmental analysis of the blood concentration—time profiles (figure 1).
[c] Compartmental analysis for the estimation of parameters using a body two-compartment model.
[d] Mean of the correlation coefficient of the compartmental fits.

range, LE is expected to be deactivated rapidly and completely into its inactive metabolites, if LE would be readily absorbed into the systemic circulation.

Metabolism and Excretion of LE After Intravenous Administration

To demonstrate the metabolism of LE in the body, firstly, the urine samples collected in the previous experiments (pharmacokinetics) were analyzed. The results indicate that urinary excretion of intact LE was about 1.5-3.5%, and the metabolite, AE, was less than 2% at the dose range of 1 to 20 mg/kg. Determination of the conjugate form of the metabolites in the urine was also carried out using β-glucuronidase and sulfatase assay as well as hydrolysis method (using 1M HCl for the detection of glycine and taurine conjugations). The results indicate that no conjugate form of the metabolites (AE or A) was present in the urine, suggesting that LE and its metabolites are also excreted through other pathways.

Therefore, studies on the biliary and urinary excretion of LE, AE and A were carried out after iv injections of LE. In figure 2, the concentration-time profiles of LE, AE and A in blood (from the pharmacokinetic study), bile and urine after intravenous administration of 10 mg/kg of LE are displayed. The metabolite, AE, appeared in blood immediately after the administration of LE with the maximal concentration observed at 3 min, and both LE and AE disappeared from the blood in about 3 hours (A in blood was not determined in the pharmacokinetic study). On the other hand, almost no LE (data not shown) but very high concentration of AE and A were found in the bile. The highest biliary concentrations of AE and A were observed shortly after LE administration. Then, AE decreased rapidly (almost parallel to that in the blood), but A remained high in bile until the end of the experiment (6 hours). Some LE and AE were excreted in the urine, but the concentrations were relatively low compared to A.

Since the elimination of LE from the blood displayed a dose-dependent kinetics, the rate of metabolism and excretion of LE could be affected by the dose administered. In table 2, the cumulative excretions of LE, AE and A in bile and urine at six hours after iv administrations of varying doses are expressed by the percentage of dose administered. It shows that only a negligible amount of LE is excreted in the bile, and the urinary excretion of LE was not dependent on the dose administered. The same phenomenon was observed in the case of the biliary and urinary excretion of AE. However, a marked dose-dependency was found in both biliary and urinary excretions of A. So that at a dose of 0.5 mg/kg, almost 100% of the drug administered was excreted from the body, and more than 96% of it was excreted as the metabolite, A.

The biliary excretions of A versus time curves are shown in figure 3. The excretion of A was relatively fast at a low dose, but it was significantly reduced at a higher dose. This dose-dependent biliary excretion of A indicates the presence of saturable enzyme system and/or saturable excretion process of A in the liver.

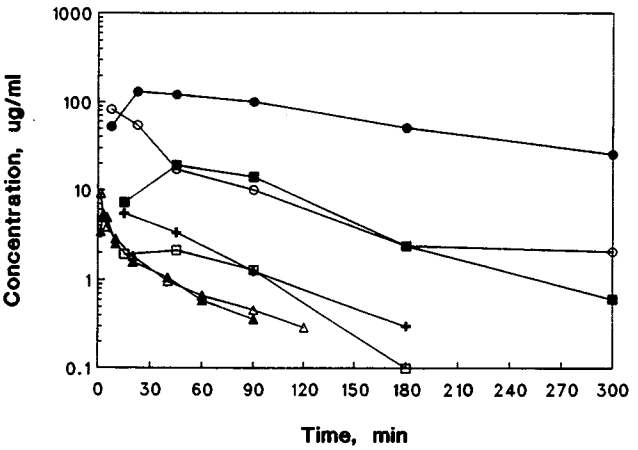

Fig. 2. Concentrations of LE, AE and A in blood, bile and urine after intravenous administration of LE at a dose of 10 mg/kg in rats. LE in blood (▲), AE in blood (△), AE in bile (○), A in bile (●), LE in urine (+), AE in urine (□), A in urine (■).

Table 2. Excretion (%) of LE and Its Metabolites, AE and A, at 6 hr after Intravenous Administration of LE in Rats

Dose, mg/kg	0.5[a]	1[a]	2[b]	5[b]	10[a]
LE bile	N.D.[c]	0.04 ± 0.01	0.05	0.08	0.10 ± 0.01
urine	1.32 ± 0.07	1.23 ± 0.26	2.76	2.38	1.82 ± 0.09
total	1.32 ± 0.07	1.27 ± 0.26	2.81	2.46	1.92 ± 0.10
AE bile	0.93 ± 0.07	1.49 ± 0.17	0.76	1.52	2.12 ± 0.31
urine	0.66 ± 0.25	0.79 ± 0.11	1.78	1.10	0.26 ± 0.05
total	1.59 ± 0.32	2.28 ± 0.28	2.54	2.62	2.38 ± 0.36
A bile	75.67 ± 0.66	44.19 ± 6.58	33.56	23.85	19.69 ± 1.13
urine	20.74 ± 1.55	17.32 ± 1.26	9.52	7.31	2.77 ± 0.69
total	96.41 ± 2.21	61.51 ± 7.84	43.08	31.16	22.46 ± 1.82
Total	99.32 ± 2.60	65.06 ± 8.38	48.43	36.24	26.76 ± 2.28

[a] Each value represents the mean ± S.E. of three to five trials.
[b] Each value represents one trial.
[c] Not detectable.

In this study, the fraction (contribution) of metabolites in the total excretion during the initial 6 hr may be a clue to predict the dose-dependent excretion of LE and its metabolites. At 6 hr after the administration, the fractions of AE and A in the total excretion, at the doses of 0.5, 1, 2, 5 and 10 mg/kg were calculated as 1.60, 3.50, 5.24, 7.23, 8.89, and 97.07, 94.54, 88.95, 85.98, 83.93, respectively. The increasing of the fraction of AE and the decreasing of the fraction of A during 6 hr is depending on the dose of LE, and showed a fairly good linear correlation against the logarithmic value of dose as shown in figure 4. The equations obtained from the linear regression for AE and A were as follows; % (AE) = 5.536 x (log dose) + 3.411 (r = 0.999), and % (A) = -10.512 x (log dose) +93.666 (r = 0.984), respectively. Using this correlation and the total excretion amounts at different doses in table 2, one may be able to predict the transformation pathway of LE to AE to A, and their apparent initial excretion rates as a function of LE dose.

LE was developed mainly for the topical use. In that case, however, the concentrations of LE and its metabolites in the systemic circulation should be much lower than the case of intravenous doses investigated in this study. The results of this study indicate that resident time of the metabolites, mainly A, in the body is remarkably depending on the dose of LE, due to the saturable excretion process. Therefore, it can be predicted that after topical administration of LE, when absorbed to the systemic circulation, will be almost completely degraded to the inactive metabolites, and the metabolites are rapidly excreted through the bile and urine, resulting in a good accordance with the soft drug concept.

These metabolism and excretion results demonstrate that, at the dose range investigated, *a)* LE is almost completely and rapidly metabolized in the central circulation and mainly in the liver; *b)* although the contribution of AE excretion is small, it was found that some saturable process may exhibit in the transformation of AE to A in the liver, since biliary excretion of AE increased with increasing dose; *c)* the final metabolite, A, is excreted into the urine and bile, and preferentially into the bile in a saturable manner.

In conclusion, present studies demonstrate the rapid deactivation as well as the controlled metabolism and excretion of LE. The results indicate that after the topical application, LE absorbed locally will rapidly undergo a predicted metabolic pathway to its inactive metabolites in the body, and the metabolites will be excreted from the body through bile and urine. So, LE can be successfully used for topical treatment of the inflammatory diseases because the unwanted systemic

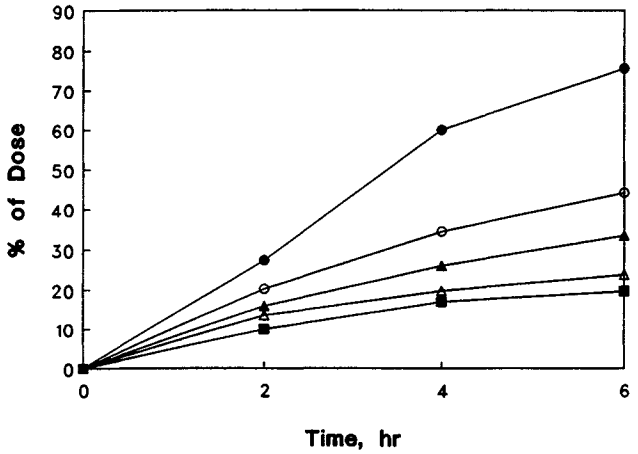

Fig. 3. Biliary excretion - time profiles of A after intravenous bolus administration of LE at various doses. 0.5 (●), 1 (○), 2 (▲), 5 (△), 10 (■) mg/kg.

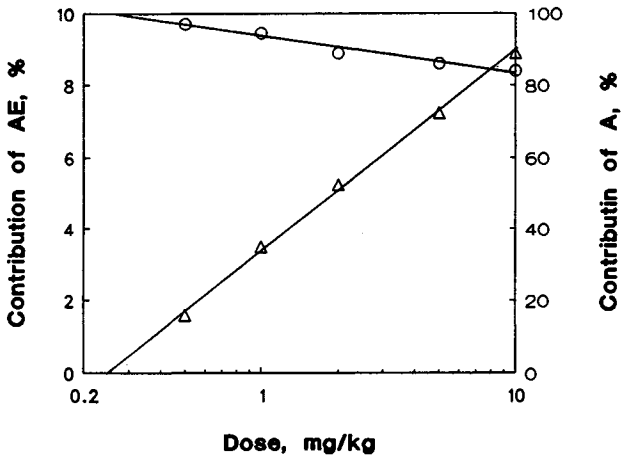

Fig. 4. Relationship between the contribution of AE and A in the total excretion and the dose of LE administered. AE (△), A (○).

side effects can be avoided after the topical pharmacological activity is achieved.

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