JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 291–297

Received August 14, 2007
Accepted November 22, 2007
DOI 10.1211/jpp.60.3.0003
ISSN 0022-3573

Pharmacokinetics of the sequential metabolites of loteprednol etabonate in rats

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Abstract

Pharmacokinetics, metabolism and excretion of two sequential inactive metabolites of the soft corticosteroid loteprednol etabonate (LE), Δ^1-cortienic acid etabonate (AE) and Δ^1-cortienic acid (A), have been investigated in rats. Pharmacokinetic studies (two-compartment model, 10 mg kg^{-1} intravenous bolus of AE or A) found the elimination of both AE ($t^1/_{2(\beta)}$, 12.46 ± 1.18 min; CL_{total}, 101.94 ± 5.80 mL min^{-1} kg^{-1}; and K_{el}, 0.24 ± 0.02 min^{-1}) and A ($t^1/_{2(\beta)}$, 14.62 ± 0.46 min; CL_{total}, 53.80 ± 1.40 mL min^{-1} kg^{-1}; and K_{el}, 0.18 ± 0.02 min^{-1}) to be significantly faster than that previously determined for the parent LE ($t^1/_{2(\beta)}$, 43.41 ± 7.58 min; CL_{total}, 67.40 ± 11.60 mL min^{-1} kg^{-1}; and K_{el}, 0.071 ± 0.024 min^{-1}). For metabolism and excretion evaluations, 1 and 10 mg kg^{-1} of either AE or A were intravenously administered, and the urine and bile were collected. AE and A rapidly reached their peak concentrations in the bile and urine, and most of them were eliminated within one hour. Total cumulative excretions at 4 h after 1 and 10 mg kg^{-1} injections were 85.51 ± 3.38% and 67.50 ± 2.67% for AE, and 71.90 ± 3.72% and 37.73 ± 2.69% for A in bile; and 4.84 ± 1.87% and 13.85 ± 3.27% for AE, and 24.28 ± 8.44% and 22.35 ± 1.12% for A in urine, respectively. After AE administration, the excretion of AE was > 90%, and A was < 10% in all cases, indicating that the elimination of AE was much faster than its metabolism (to A). In a manner similar to that seen for LE, dose-dependent elimination was observed both in AE and A. These results suggested that both AE and A were ideal leads for the design of soft steroids based on the inactive metabolite approach.

Introduction

During the design and selection of new therapeutic agents, the main focus should not be solely on the increase of the therapeutic activity, but also on the improvement of safety to achieve a higher therapeutic index (TI). Many widely used drugs, including some that are used for treating local symptoms, have multiple activity, and they may cause many undesired, mostly receptor-mediated, systemic, toxic side-effects. The concept of soft drugs provides specific methods for introducing structure–metabolic relationships into the drug design process, and it makes possible the design and prediction of the major metabolic pathway of a drug. Thus, with soft drugs one can avoid the formation of toxic/undesired intermediates, and increase the TI (Bodor 1984, 1991). Loteprednol etabonate (chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1, 4-diene; LE) was designed based on this soft drug concept within the framework of retrometabolism-based drug design approaches (Bodor 1988, 1993; Bodor & Buchwald 2002, 2006). LE showed potent local anti-inflammatory effects (similar to dexamethasone) with a good systemic safety profile and a significant improvement in the therapeutic index (TI = 24) compared with other, classical corticosteroids (TI = 1.0–1.5) (Bodor & Varga 1990; Druzgala et al 1991a, b; Bodor et al 1992a; Howes & Novack 1998). It received FDA approval as an ophthalmic anti-inflammatory agent for the treatment of inflammation following eye surgery as well as allergic conjunctivitis (Howes 2000).

LE has a biologically labile 17β-chloromethyl ester function designed to undergo rapid systemic hydrolysis to 17β-carboxylate, resulting in Δ^1-cortienic acid etabonate (AE); further hydrolysis leads to Δ^1-cortienic acid (A), as shown in Figure 1, both being inactive

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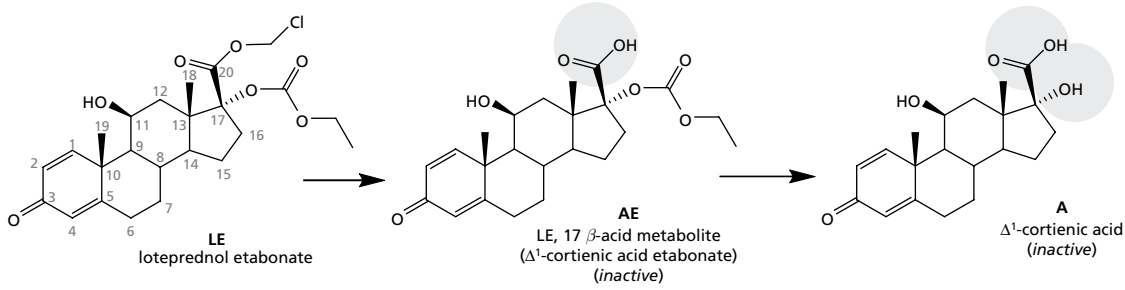

Figure 1 Metabolic pathway of loteprednol etabonate.

metabolites. This predictable and facile metabolic inactivation was introduced to optimize local efficacy and to reduce systemic activity. After oral administration to dogs and rats, only inactive metabolites and no intact LE was detected in plasma (Hochhaus et al 1992). In rabbit eye, the predicted conversion of the active soft steroid to the inactive metabolites was observed in all compartments of the eye (Druzgala et al 1991b). Detailed pharmacokinetic, metabolism, distribution, and excretion studies of LE revealed that after intravenous injection of LE in rats, the majority of the parent compound (LE) indeed went through the two-step sequential metabolism shown in Figure 1 in-vivo, first to AE and subsequently to A (Bodor et al 1992b). A dose-dependent disposition of LE was observed for the dose range of 1–20 mg kg^{-1} (i.v.), and it was believed to be due to the saturable metabolism of LE as well as saturable elimination of the inactive metabolites into the bile and urine (Bodor et al 1995b). It has been shown that both metabolites, AE and A, have no glucocorticoid receptor binding activity; thus, no steroid side effects are expected for them (Druzgala 1991a). With the possibility of extending the clinical applications of soft corticosteroids to other therapeutic areas, such as treatment of colitis (Bodor et al 1995a), rheumatoid arthritis (Buris et al 1999), or asthma (Hochhaus & Derendorf 1995), and the ongoing attempts at designing more potent and safer soft corticosteroids based on the concept of inactive metabolites, we felt it was important to characterize, in detail, the pharmacokinetics properties of the inactive metabolites, AE and A.

Materials and Methods

Materials

Loteprednol etabonate (LE) was obtained from Otsuka Pharmaceutical Co., Ltd (Tokushima, Japan). Δ^1-Cortienic acid etabonate (AE) and Δ^1-cortienic acid (A) were from Xenon Vision Inc. (Alachua, FL). Hydroxypropyl-β-cyclodextrin (HP-β-CyD) was obtained from Pharmatec, Inc. (Alachua, FL). All other chemicals were commercially available products of special reagent grade. Male Sprague-Dawley rats (250–300 g) were obtained from Charles Rivers (Wilmington, MA). Animal studies were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals adopted by the National Institutes of Health, and approved by the Institutional Animal Care and Use Committee at the University of Florida.

Intravenous administration of AE and A

Rats (250–300 g) were anaesthetized with an injection of sodium pentobarbital (30 mg kg^{-1}, i.p.), and the jugular vein was exposed. A and AE were dissolved in a 50% HP-β-CyD aqueous solution and injected into a jugular vein over 1 min at a dose of 10 mg kg^{-1} and a dosing volume of 1 mL kg^{-1}. Subsequently, blood samples (0.1 mL) were collected through the fellow jugular vein at appropriate time intervals for 90 min. The samples were added to 0.2 mL acetonitrile containing 5% dimethyl sulfoxide (DMSO) solution, mixed with a vortex mixer, and centrifuged at 10 000 rev min^{-1} for 1 min. The supernatant was then analysed by HPLC. The recovery of AE and A was $100 \pm 3\%$ with this method.

Pharmacokinetics

Non-compartmental and compartmental pharmacokinetic analyses were performed. For non-compartmental analysis, the area under the curve (AUC) of the plasma concentration vs time was calculated using the linear trapezoidal rule, and the area from the last measurement, C_t, to infinity was calculated as C_t/β, where β was the terminal disposition rate constant; AUMC, the area under the first moment curve, was calculated using the trapezoidal rule from the curve of blood concentration $\times$ time–time, and the area from the last time point, t, to infinity was calculated as $C_t/\beta + C_t/\beta^2$. The total body clearance (CL_{tot}) was calculated as Dose/AUC. Mean resident time (MRT) was calculated as AUMC/AUC. The volume of distribution at steady state (Vd_{ss}) was determined as the product of CL_{tot} and MRT.

Compartmental pharmacokinetic analysis was performed with PK-Analyst (MicroMath, Salt Lake City, UT). Best fit was obtained with an empirical two-compartment model, $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C was the drug concentration in blood, A and B were the exponential multipliers, α and β were the hybrid constants in the central and peripheral compartment, respectively. The volume of distribution of the central compartment (Vd_c) and the volume of distribution during the elimination phase (Vd_{area}) were calculated as Dose/(A + B) and CL_{tot}/β, respectively. The AUC was determined as $A/\alpha + B/\beta$, the half-life of the terminal phase, $t^1/_2$,

was calculated as $\ln 2/\beta$, and the elimination rate constant (K_{el}) was calculated as CL_{tot}/Vd_c.

Metabolism and excretion of AE and A after intravenous administration

Rats (250–300 g) were anaesthetized with an injection of sodium pentobarbital (30 mg kg^{-1}, i.p.). The urinary tract was closed to prevent urination, and the urine samples were collected directly from the urinary bladder through a 26-gauge needle. The common bile duct was cannulated using polyethylene tubing (PE 10, Clay Adams). AE and A solutions were administered intravenously as described before at a dose of 1 or 10 mg kg^{-1}. Total bile and urine were collected at varying time intervals (e.g. 0.5, 1, 1.5, 2, 3, and 4 h). The bile and urine samples (0.1 mL) were added to 0.1 mL 0.1 M HC1 and 1 mL ethyl acetate, and mixed vigorously by a vortex mixer for 3 min. After centrifugation at 10 000 rev min^{-1} for 1 min, 0.8 mL of the upper organic layer was separated. Subsequently, the organic solvent was evaporated in a vacuum centrifuge, reconstituted in 0.3 mL 30% acetonitrile solution, and 20 μL of the resulting solution was injected in the HPLC.

The pharmacokinetic and excretion results of LE used here were adapted from a published study (Bodor et al 1995b). That study used an experimental design in terms of animals (body weight, age, sex), sampling (blood, bile, and urine), and analytical methods similar to those reported here, and the experiments were performed by some of the same people. Therefore, data should be consistent enough to be comparable with that obtained here for AE and A.

Analytical methods

An HPLC system operating at ambient temperature was used for quantitative determination of AE and A. A Waters Nova-Pak Radial-Pak cartridge (4 mm, 5 mm × 10 cm) was connected to a component system from Spectra-Physics, which consisted of a SP 8810 precision isocratic pump, Rheodyne 7125 injector (injection volume 20 μL), SP 8450 UV/vis variable wavelength detector operated at 254 nm, and a SP 4290 integrator. At a flow rate of 1 mL min^{-1}, a mobile phase consisting of acetonitrile, acetic acid and water in a volume ratio of 60:0.2:40 eluted AE and A at 4.35 and 3.25 min, and a mobile phase consisting of acetonitrile, acetic acid and water in a volume ratio of 35:0.1:65 eluted AE and A at 18.65 and 5.85 min, respectively. The detection limit was less than 0.1 μg mL^{-1} for A and AE. Standard curves of AE and A were developed by adding known amounts of the compound to blank blood, urine 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 ($r = 0.999$).

Statistical analysis

All data were obtained as four to six replicates. Differences in the pharmacokinetic parameters of the three compounds (Table 1) have been compared using the Kruskal–Wallis test as a suitable non-parametric test to compare multiple means (Jones 2002). Differences in the cumulative eliminations (bile vs urine) for the two compounds (AE and A) at the various doses (1 and 10 mg) and collection time-points (1 and 4 h) studied (Tables 2, 3) have been compared using one-way analysis of variance followed by Tukey's HSD test as a suitable post-hoc test for individual differences (Jones 2002). A significance level of $P < 0.05$ was used in all cases. Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

Results and Discussion

Pharmacokinetics

Figure 2 shows the blood LE (Bodor et al 1995b), AE, and A concentration vs time curves following intravenous administration of 10 mg kg^{-1} of each compound itself in rats. Blood AE and A concentrations declined rapidly in a bi-exponential manner. They fell below the detection limits after 60 and 40 min for AE and A, respectively, suggesting a rapid distribution and elimination for both AE and A. The corresponding pharmacokinetic parameters derived from compartmental and non-compartmental analysis are shown in Table 1. The concentration–time curves of AE and A could be adequately described by an empirical two-compartmental model, $C = Ae^{-\alpha t} + Be^{-\beta t}$, with a good correlation coefficient of the compartmental fits (> 0.995). The values of Vd_{ss} for AE and A were 930 ± 65 and 799 ± 21 mL kg^{-1}, respectively, both being very close to the value of extracellular water content (the body-water content, 701 mL kg^{-1}) in rats (Gerlowski & Jain 1983). This suggested that the distribution of the inactive metabolites was confined to the extracellular water compartment and that almost no intercellular distribution occurred. However, the Vd_{ss} obtained for the parent compound LE (3078 ± 79 mL kg^{-1}) was more than four-times higher than the total body-water content, indicating that after administration, a significant portion of LE was intracellularly distributed and entrapped in the local tissue where it could exert its anti-inflammatory effect. Subsequently, LE was hydrolysed into the inactive metabolites AE and A by ubiquitous esterase according to the principles of soft drug design (Bodor et al 1992b, 1995b; Bodor & Buchwald 2000). Due to their considerably more polar character as compared with LE, the hydrolysed metabolites, AE and A, in the extracellular compartment were rapidly eliminated from the systemic circulation, while the elimination of the metabolites formed intracellularly should have been slower. This was in agreement with the excretion results of LE reported by Bodor et al (1995b), where at 6 h after LE administration (0.5–10 mg kg^{-1}), both dose-dependent elimination and saturable excretion ($99.32 \pm 2.60\%$ to $26.76 \pm 2.28\%$) were observed, and the inactive metabolite A was the main metabolite excreted ($96.41 \pm 2.21\%$ to $22.46 \pm 1.82\%$). Accordingly, the local pharmacological activity of LE was preserved, while systemic toxicity was avoided.

At the dose of 10 mg kg^{-1}, the total clearance CL_{tot} of AE (101.9 ± 5.8 mL min^{-1} kg^{-1}) was significantly ($P < 0.05$) larger than that of LE (67.4 ± 11.6 mL min^{-1} kg^{-1}). Both

Table 1 Pharmacokinetics of LE, AE and A after intravenous bolus injection of each compound itself in rats

	LE[a,b]	AE[b]	A[b]
Dose ($mg\,kg^{-1}$)	10	10	10
AUC ($\mu g.min\ mL^{-1}$)[c]	159.2 ± 31.3	99.1 ± 5.7	186.3 ± 5.0
CL_{tot} ($mL\ min^{-1}\,kg^{-1}$)[c]	67.4 ± 11.6	101.9 ± 5.8	53.8 ± 1.4
MRT (min)[c]	48.72 ± 8.95	9.11 ± 0.27	14.87 ± 0.44
Vd_{ss} ($mL\ kg^{-1}$)[c]	3078 ± 79	930 ± 65	799 ± 21
A ($\mu g\ mL^{-1}$)	8.65 ± 2.75	22.39 ± 3.18	33.50 ± 5.75
B ($\mu g\ mL^{-1}$)	1.92 ± 0.25	2.58 ± 1.16	6.08 ± 0.66
α ($1\,min^{-1}$)[d]	0.28 ± 0.15	0.41 ± 0.07	0.56 ± 0.07
β ($1\,min^{-1}$)[d]	0.017 ± 0.003	0.057 ± 0.005	0.048 ± 0.003
$t^1/_{2(\beta)}$ (min)[d]	43.41 ± 7.58	12.46 ± 1.18	14.62 ± 0.46
Vd_c ($mL\,kg^{-1}$)[d]	1092 ± 264	437 ± 51	307 ± 31
Vd_{area} ($mL\,kg^{-1}$)[d]	3987 ± 289	1851 ± 261	1139 ± 64
K_{el} ($1\,mL^{-1}$)[d]	0.071 ± 0.024	0.236 ± 0.018	0.182 ± 0.021
r[e]	0.998 ± 0.001	0.996 ± 0.001	0.998 ± 0.001

[a]Data adapted from previous studies (Bodor et al 1995b). [b]Data represent mean ± s.e. of four trials. [c]Parameters obtained by noncompartmental analysis of the blood concentration–time profiles. [d]Compartmental analysis for the estimation of parameters using a body two-compartment model. [e]Correlation coefficient of the compartmental fits.

Table 2 Cumulative elimination (% of dose) after intravenous administration of AE to rats[a]

Dose	$10\,mg\,kg^{-1}$		$1\,mg\,kg^{-1}$	
	1 h	4 h	1 h	4 h
AE				
Bile	59.73 ± 0.72	62.53 ± 1.95	73.73 ± 4.30	79.79 ± 1.86
Urine	10.00 ± 2.68	11.62 ± 2.43	3.58 ± 0.90	3.94 ± 1.04
Subtotal	69.73 ± 3.13	74.15 ± 1.44	77.31 ± 3.40	83.73 ± 2.91
A				
Bile	3.68 ± 0.54	4.97 ± 0.72	3.67 ± 1.00	5.72 ± 1.52
Urine	1.74 ± 0.96	2.23 ± 0.84	0.67 ± 0.12	0.90 ± 0.01
Subtotal	5.43 ± 1.30	7.20 ± 0.88	4.38 ± 0.94	6.61 ± 1.51
AE + A				
Total	75.16 ± 3.69	81.35 ± 0.75	81.68 ± 4.34	90.34 ± 1.39

[a]Data represent mean ± s.d. of 4–6 trials.

Table 3 Cumulative elimination (% of dose) after intravenous administration of A to rats[a]

Dose	$10\,mg\,kg^{-1}$		$1\,mg\,kg^{-1}$	
	1 h	4 h	1 h	4 h
Bile	35.65 ± 2.49	37.73 ± 2.69	59.63 ± 3.25	71.90 ± 3.72
Urine	16.54 ± 3.47	22.35 ± 1.12	23.23 ± 7.86	24.28 ± 8.44
Total	52.18 ± 5.48	60.08 ± 3.01	82.86 ± 4.60	96.18 ± 4.72

[a]Data represent mean ± s.d. of 4–6 trials.

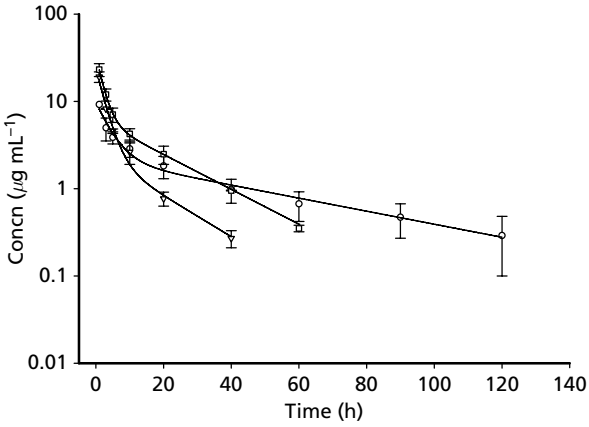

Figure 2 Blood concentration–time profiles of LE (○), AE (▽), and A (□) in rats after intravenous bolus injection of each compound itself at a dose of 10 mg kg^{-1}. Each mark represents mean ± s.e. of four data.

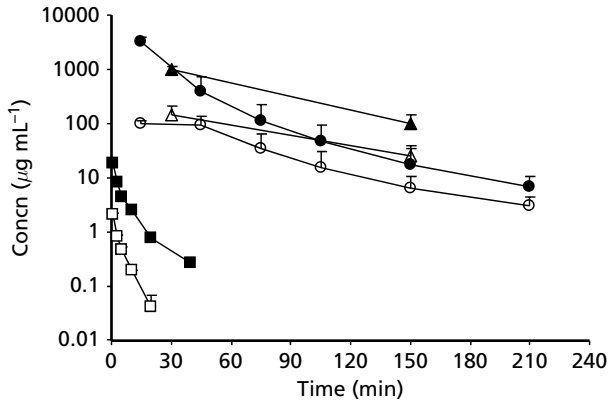

Figure 3 Blood, bile and urine concentration–time profiles after intravenous injection of 10 mg kg^{-1} AE in rats. (■) AE in blood, (□) A in blood, (●) AE in bile, (○) A in bile, (▲) AE in urine, (Δ) A in urine. Each mark represents mean ± s.d. of six data.

values were higher than the physiological hepatic blood flow, 58 mL min^{-1} kg^{-1} in rats, indicating that in addition to the metabolism in liver, enzymatic degradation of LE and AE also took place in the blood and in other organs, such as lung, intestine and kidney, that played a significant role in the systemic elimination of LE and AE. The CL_{tot} of A (53.8 ± 1.4 mL min^{-1} kg^{-1}) was similar or maybe slightly less than the physiological hepatic blood flow, indicating that clearance of A from the body may have been more sensitive to hepatic saturation than that of AE and LE. The larger values of the elimination rate constants of AE (0.236 ± 0.018 min^{-1}) and A (0.182 ± 0.021 min^{-1}) compared with that of LE (0.071 ± 0.024 min^{-1}) indicated a faster elimination of the inactive metabolites (AE and A) than that of the parent soft drug, LE. These results were consistent with the more polar, more hydrophilic nature of AE and A, as compared with LE, making them much more likely to be eliminated faster than LE.

Metabolism and excretion

Figure 3 shows the concentration–time profiles of AE and A in blood, bile and urine after intravenous administration of 10 mg kg^{-1} AE in rats. The metabolite A was immediately detected in the blood with the maximum concentration observed at 1 min after AE injection, suggesting that hydrolysis of AE to A occurred rapidly in-vivo. The concentration of A in blood was approximately one-tenth of AE during the first 5 min and decreased to approximately one-twentieth of AE by 20 min. Metabolite A was then rapidly eliminated ($t^1/_2$ = 4.06 min) from the blood along with AE ($t^1/_2$ = 12.46 min). In bile, both AE and A appeared shortly after AE injection, and the concentration of A reached a plateau within less than 15 min. The concentrations of both AE and A declined then with K_e rates of 0.0521 min^{-1} and 0.0368 min^{-1}, respectively (corresponding to $t^1/_2$ values of 13.30 min and 18.83 min). These results, again, indicated a rapid excretion of AE and A from the systemic circulation to the bile. AE and A also appeared early in urine. Although the concentrations of AE and A remained high due to low urine flow rate, the cumulative elimination in urine was much lower than in bile.

The cumulative elimination (% dose) of AE and A in bile and urine at 1 and 4 h after intravenous injection of AE are shown in Table 2. Following AE administration (1 or 10 mg kg^{-1}), totals (AE + A) of 90.34% (85.51% from bile, 4.84% from urine) and 81.35% (67.50% from bile, 13.85% from urine) were recovered, respectively. The significantly ($P < 0.05$) higher excretions in the bile indicated that biliary excretion was the main route of elimination for AE and A. Results indicated that the ratio of biliary to urinary excretion as well as the ratio of AE to A excretion were both higher after 1 mg kg^{-1} administration than after 10 mg kg^{-1} administration. Hence, a saturable metabolism/elimination process in the liver might have been involved in the excretion of AE and A. This was in agreement with the previous observations for LE excretion (Bodor et al 1995b). The dose-dependent process could be due either to the transport of AE and A across the bile canalicular membrane or to the conversion of AE into A. Under clinical settings, only much lower doses of LE are administered (e.g. ophthalmic, dermal or other topical applications), and, therefore, the concentrations of LE and its metabolites, AE and A, will be far below the levels where such saturation seems to be present, resulting in fast, unlimited eliminations. The total excretion (AE and A in bile and urine) at 1 h after 1 and 10 mg kg^{-1} administration of AE were 81.68% and 75.16%, respectively, reinforcing again the observation that both inactive metabolites of LE were highly polar and subject to fast elimination from the systemic circulation. After intravenous injection, the majority (> 90%) of the administered AE was eliminated as the unchanged form in all cases, indicating that the rate of AE to A conversion was much less than that of AE excretion due to the hepatic and renal clearance of AE being much larger than the metabolic clearance of AE. In a separate study in two rats, bile and urine samples were taken at 6 h after intravenous administration of 10 mg kg^{-1} AE. At 6 h, mean biliary and urinary excretion values of 86.0% (74.2% of AE, 11.8% of A) and 13.4% (9.4% of AE, 3.9% of A), respectively, were found,

indicating that excretion could be almost completed (99.4%) at 6 h after administration.

Finally, the concentration–time profiles of A in blood, bile and urine after intravenous administration of 10 mg kg^{-1} of A are shown in Figure 4. Similar to the case of AE, A appeared in the bile and reached peak concentration shortly after intravenous injection; then, the concentration declined fairly rapidly at a similar rate ($t^1/_2 = 15.46$ min) to that of the blood elimination ($t^1/_2 = 14.62$ min). In the urine, the concentration of A was about the same as in the bile at 30 min, and was still relatively high at 150 min – approximately the same as in the case of AE. The excretion rate of A in both bile and urine was somewhat less than that of AE ($t^1/_2$, 15.46 min vs 13.30 min, respectively), in agreement with previous pharmacokinetic results. The cumulative excretion (% of dose) of A in the bile and urine after intravenous injections are summarized in Table 3. Similar to AE, A was mainly excreted in the bile. At 4 h after 1 and 10 mg kg^{-1}, bile excretions were 71.90% and 37.73%, and urine excretions were 24.28% and 22.35%, respectively. Urinary excretion seemed to play a more important role in the elimination of A than in the elimination of AE (24.28% and 22.35% vs 4.84% and 13.85%, respectively). Furthermore, a stronger dose-dependency was observed for the elimination of A than for that of AE. At 4 h after intravenous injection of 1 and 10 mg kg^{-1}, the total excretions of A were 96.18% and 60.08%, whereas those of AE were 90.34% and 81.35%, respectively (with similar results at 1 h). Thus, the saturation phenomenon seen for A in the LE excretion study (Bodor et al 1995b) was probably due to both the saturable metabolisms (LE to AE and AE to A) and the saturable elimination of A itself.

Comparison of these results with those obtained for AE and A after LE administration indicated that AE and A followed a similar dose-dependent elimination pattern in both cases. However, after a higher dose, such as 10 mg kg^{-1} AE or A alone, a majority of the dose administered was excreted in the bile and urine after 4 h (81.35 ± 0.75% and 60.0 ± 3.01%, respectively), while after 10 mg kg^{-1} LE, only 23.91 ± 2.06% was excreted (mainly in the form of A), suggesting that the distribution and metabolism processes of LE could be the main factors delaying the excretion. At a lower dose (1 mg kg^{-1}) of AE and A, most excretion was completed in 4 h (90.34 ± 1.39% and 96.18 ± 4.72%, respectively), and, after the same dose of LE, the total excretion (55.42 ± 8.22%) was largely increased. When the dose of LE was further reduced to 0.5 mg kg^{-1}, the total LE excretion was further increased to 83.64 ± 2.98%. This clearly indicated that after LE administration, both dose-dependent formation and elimination of the metabolites occurred, and that the inactive metabolites, by themselves, could be rapidly eliminated from the body. Thus, a clearer pharmacokinetic picture of the events occurring after LE administration has been obtained.

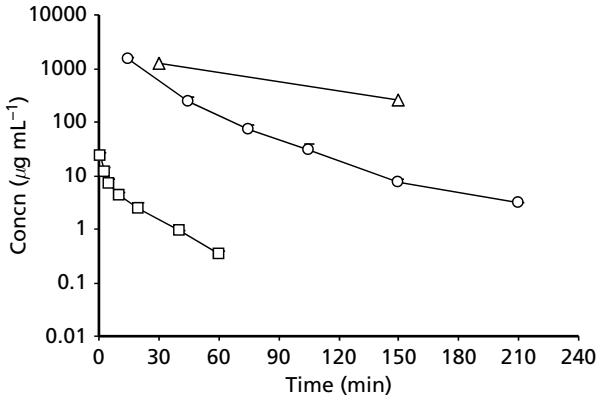

Figure 4 Blood, bile, and urine concentration–time profiles after intravenous injection of 10 mg kg^{-1} A in rats. (□) A in blood, (○) A in bile, (Δ) A in urine. Each mark represents mean ± s.d. of four data.

Conclusion

The pharmacokinetics, metabolism and elimination of AE and A after single intravenous injection has been studied and compared with that of the fate of AE and A after LE administration, the clinical setting of interest. Pharmacokinetic results indicated that systemically administered AE and A were both primarily contained in the extracellular water (body-water) compartment and subject to rapid elimination via biliary (major) and urinary (minor) excretion. The metabolism of AE to A, and the elimination of AE and A were both dose-dependent. Considering the concept of inactive metabolite-based soft drug approach, these pharmacokinetic properties together with the lack of pharmacological activity of these compounds make AE and A ideal lead compounds for the design of soft corticosteroids.

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