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The Physicochemical Properties, in Vitro Metabolism and Pharmacokinetics of a Novel Ester Prodrug of EXP3174

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Abstract: EXP3174 is the major active metabolite of losartan, a drug currently widely used for the treatment of cardiovascular diseases. This study was designed to evaluate the physicochemical properties of EXP3174-pivoxil (a novel synthesized prodrug of EXP3174) and characterize its metabolism, regional intestinal absorption and pharmacokinetics by in vitro and in vivo studies. An in vitro metabolism study was conducted in liver and intestinal S9 fractions from different species including rat, dog and human. In vivo absorption was investigated following regional intestinal dosing in rats, and the pharmacokinetics was determined using rats after a single oral administration. EXP3174-pivoxil exhibited predictable stability in the aqueous solution within a pH range of 1.2−9.0 as well as in the solid form of powder. An in vitro metabolism study revealed that EXP3174-pivoxil was rapidly and efficiently converted into EXP3174 by enzymatic hydrolysis. The dose administered into the duodenum and jejunum resulted in higher values for the AUC_{0-24h} and C_{max} than those following ileum dosing ($p < 0.05$). Furthermore, the AUC_{0-24h} and C_{max} values for EXP3174 increased in a dose-dependent manner as dose increased from 0.5 to 5 mg/kg. A comparable AUC_{0-24h}, shortened T_{max} and a significant increase in the plasma C_{max} of EXP3174 were observed following oral administration of EXP3174-pivoxil (as EXP3174, 1 mg/kg) compared with those of losartan (as EXP3174, 5 mg/kg) in rats, suggesting faster absorption and a 5-fold enhancement in the bioavailability of EXP3174. These results suggest that EXP3174-pivoxil may serve as a more effective drug even at lower clinical doses by exhibiting increased bioavailability and faster therapeutic response, compared with losartan.

Keywords: Prodrug; EXP3174; physicochemical property; metabolism; pharmacokinetics; oral bioavailability

Introduction

Losartan is a prototype angiotensin II AT_1- receptor antagonist and is a well-accepted agent for the treatment of hypertension and other cardiovascular diseases in the clinic.[1,2] However, EXP3174, the major active metabolite of losartan in the liver, is primarily responsible for the therapeutic response.[3] EXP3174 is 10- to 40-fold more potent than losartan and has a longer duration of action than losartan.[4,5]

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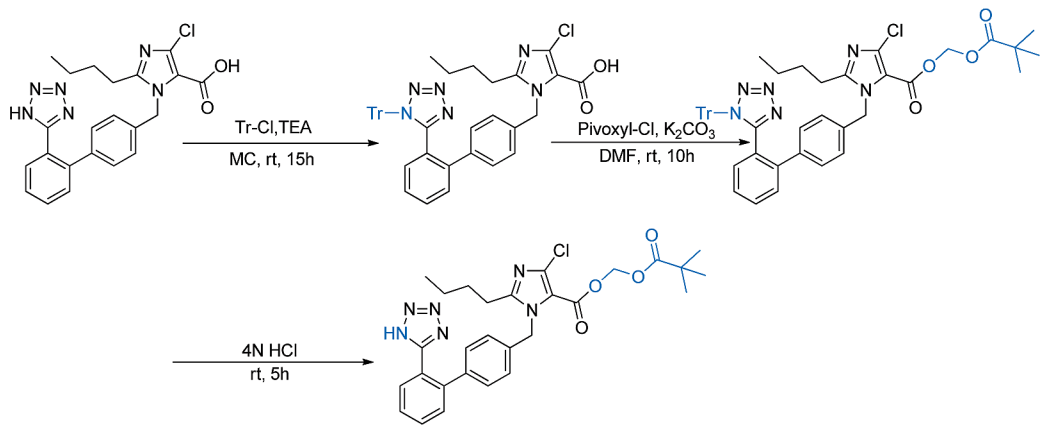

Figure 1. Synthesis of the ester prodrug of EXP3174-pivoxil.

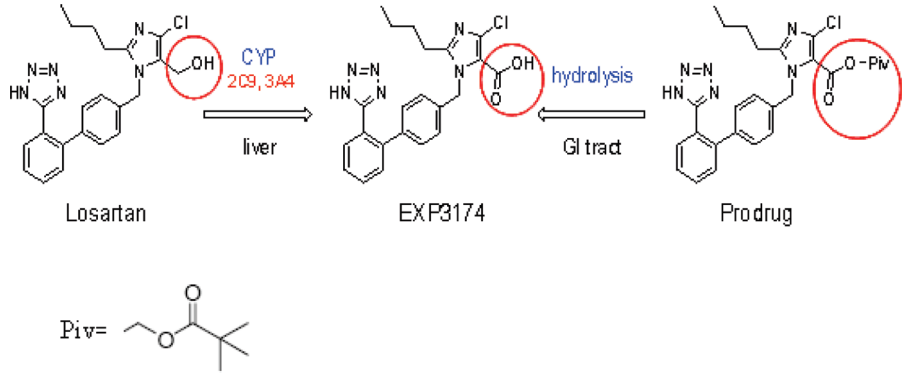

Figure 2. Transformation of losartan and EXP3174-pivoxil into EXP3174.

Unfortunately, the generation of EXP3174 from losartan depends on the oxidase activity of metabolic enzymes (CYP 3A4 and 2C9 isoenzymes) in the individual, which may reduce the predictability of efficacy and side effects.[6]

Recently, considerable attention has been focused on the development of prodrugs, which provides an effective strategy for improving the pharmaceutical, pharmacokinetic and pharmacodynamic characteristics of a therapeutic agent.[7] Ester formation represents a well-known approach. Approximately 49% of all marketed prodrugs are activated by hydrolysis. Currently, ester prodrugs are the predominant types of prodrugs.[8,9] The formation of the ester is intended to increase the overall lipophilicity of the molecule and promote membrane permeability and oral absorption, which mostly leads to major improvements in oral bioavailability.[10]

Pivalate has been used successfully to generate ester prodrugs to increase oral bioavailability (for example cefetamet pivoxil, pivampicillin,[11] cefditoren pivoxil,[12] and adefovir dipivoxil[13]). Although long-lasting therapy of a pivalate-generating prodrug may inevitably result in perturbed carnitine homeostasis,[14] it has also been reported that the extent of tissue carnitine depletion is dependent on the dose of pivalate. The release of pivalic acid is not a concern given that the daily pivalate load from the prodrug at a low level would not adversely affect carnitine metabolism due to the large body carnitine.[15] This assumption is supported by the approval of adefovir dipivoxil for chronic treatment of hepatitis B. With this regard, a novel compound, EXP3174-pivoxil, {2-butyl-4-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-imidazole-5-carboxylic acid pivaloyloxymethyl ester}, was synthesized based on preliminary screening

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(as shown in Figure 1). This compound was designed to be converted into EXP3174 by hydrolysis rather than oxidation (Figure 2). It was expected that the generation of EXP3174 from EXP3174-pivoxil might be much more uniform and faster than that of losartan since the process of hydrolysis is much easier to trigger and is less affected by individual differences in metabolic enzyme activity compared with that of oxidation.[7,10,16]

The present work was initiated to develop a novel prodrug of EXP3174 possessing highly predictable efficacy and favorable physicochemical properties. EXP3174-pivoxil was chosen, and its solubility, pH stability and powder stability in stress conditions were investigated. Its in vitro metabolism was assessed in liver and intestinal S9 fractions from rat, dog and human. Both in vivo absorption and pharmacokinetics were determined in rats using losartan as a comparative reference drug in order to gain insight into the clinical potential of the EXP3174-pivoxil.

Experimental Section

Materials. EXP3174 and EXP3174-pivoxil were synthesized by the research center at Hanmi Pharm. Co. (Hwaseung, South Korea). Losartan and candesartan were obtained from Hanmi Pharm. Co. (Hwaseung, South Korea). Acetonitrile was of high-performance liquid chromatography (HPLC) grade. All other reagents were of analytical reagent grade.

Animals. Male Sprague−Dawley rats weighing 250 ± 20 g for the regional intestinal absorption study were purchased from Charles River Company Korea (Orient, Seoul, South Korea). Male Sprague−Dawley rats (6−7 weeks old) weighing between 180 and 200 g for the in vivo pharmacokinetics study were obtained from Samtaco Bio Korea Inc. (Korea). All animal care practices and experimental procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology.[17] The protocols for the animal studies were also approved by the Institute of Laboratory Animal Resources of Yeungnam University. All rats had free access to tap water and a pellet diet. The animals were housed in a cage and maintained in a 12 h light/dark cycle at 25 °C/55 ± 10% RH. General and environmental conditions were strictly monitored.

Physicochemical Properties. *(A) Aqueous Solubility and pH Stability.* Aqueous solubility studies were carried out in pH 1.2 and 6.8 buffer systems and distilled water. An excess amount of drug was added to 1 mL of buffer or water in glass vials and placed in a shaking water bath at 25 °C and 60 rpm for 24 h. At the end of this time period, solutions were centrifuged at 12500*g* for 10 min and the supernatant was filtered through a 0.45 μm syringe filter membrane. The samples were analyzed within 15−20 min of taking the sample to prevent any precipitation. The initial 0.5 mL of the filtrate was discarded as an added precaution to avoid adsorption of EXP3174-pivoxil onto the filter membrane. Solubility studies were carried out in triplicate. The drug concentration was determined using an HPLC method on a Waters Alliance HT Chromatography System (Waters Corp., Milford, MA, USA), with a Nucleosil column (C_{18}, 4.0 × 250 mm, 5 μm). The column temperature was held at 35 °C. The mixing solution of acetonitrile and 0.1% phosphoric acid (40:60, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min with a run time of 30 min. The retention times were about 8.7 min, 12.5 min, and 24.2 min for EXP3174, losartan, and EXP3174-pivoxil, respectively. The injection volume was 20 μL, and ultraviolet detection was set at 254 nm.

The pH stability of the EXP3174-pivoxil was investigated by measuring the purity (HPLC area %) of EXP3174-pivoxil at pH 1.2, 4.0, 6.8 and 9.0. The samples were taken out at 0, 1, 6, 12, 18 and 24 h after storage at 37 °C and analyzed by HPLC, as mentioned above.

(B) Powder Stability Test. To evaluate the stability of EXP3174-pivoxil powder, the degradation compounds, water content and amount remaining after storage were examined. For the accelerated and stressed stability test, the powder was kept in the glass vial with or without a rubber stopper and stored in three conditions, i.e., at 40 °C/75% RH, 25 °C/60% RH and 60 °C/75% RH for 28 days. The water content was measured by the dry loss method. Furthermore, the amount and purity of EXP3174-pivoxil were analyzed by HPLC. All samples were tested in triplicate.

In Vitro Metabolism in Liver and Intestinal S9 Fraction. The in vitro metabolism of EXP3174-pivoxil or losartan was investigated in incubations with S9 subcellular fractions from rat, dog, and human liver homogenates and intestinal homogenates (Xenotech, Lenexa, KS).[18,19] The rate of metabolism was measured in triplicate under the following conditions: EXP3174-pivoxil or losartan in DMSO (0.5 mM); S9 fractions (20 μL, 1 mg/mL); pH 7.4 sodium phosphate

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buffer (56 mM). Incubations were performed in 96-well plates at 37 °C under an atmosphere of 5% CO_2. The reactions were started by the addition of 20 μL of EXP3174-pivoxil stock solution to make a final concentration of 50 μmol. At predetermined intervals, the reactions were stopped by adding 200 μL of ice-cold acetonitrile to each well. The samples were centrifuged at 12000*g* for 10 min, and the amount of remaining parent drugs and formed EXP3174 in the supernatant was measured by HPLC.

In Vivo Absorption Studies. *(A) Regional Intestinal Absorption Study in Rats.* Thirty male Sprague−Dawley rats were randomly divided into six groups ($n = 5$ for each group). The rats were anesthetized with pentobarbital (50 mg/kg ip). A midline abdominal incision was made, and the intestinal segments to be perfused were identified.[20,21] EXP3174-pivoxil or losartan dissolved in a mixture of PEG400/Tween80/ethanol/water (8:1:1:90) was administered as a single bolus dose of 5 mg/kg (as EXP3174) through the catheter placed in the duodenum, the jejunum, or the ileum, respectively. The duodenal doses and the jejunal dose were given approximately 4−5 and 30 cm distal to the pylorus at a volume of 1.5 mL/kg, respectively, and the ileal dose was given approximately 20 cm proximal to the cecum. Immediately after administration, the catheters were flushed with 250 μL of the solution. Blood samples were collected from the right or left subclavian vein or artery at 0 (prior to dosing) and 0.5, 1, 2, 3, 4, 6, 10 and 24 h after dosing.

(B) In Vivo Pharmacokinetics in Rats. The rats were fasted for 24 h prior to the experiments with free access to water. Twenty-five male Sprague−Dawley rats were divided into five groups. The rats in the first group were orally administered 1 mL of 0.5% CMC aqueous suspension containing losartan at a dose of 5.0 mg/kg (as EXP3174). In a similar manner, those in the remaining four groups were given EXP3174-pivoxil at a dose of 0.5, 1.0, 2.5 and 5.0 mg/kg (as EXP3174), respectively. Blood samples were collected from the right or left subclavian vein at 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h after administration. Blood samples were held on ice until centrifugation at 10000*g* for 10 min. Plasma was then stored at −20 °C until analysis.

Determination of Losartan and EXP3174 in Rat Plasma. Two hundred microliters of 0.1 M citric acid, 10 μL of candesartan standard solution (500 ng/mL) and 1 mL of MTBE were added to 100 μL of rat plasma samples. The resultant mixture was vortexed for 1 min and centrifuged at 1400*g* for 10 min. The supernatant was transferred to a 15 mL centrifuge tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 50 μL of the mobile phase.

All samples obtained from in vivo studies were assayed using HPLC−MS.[22,23] The Waters 2795 HT chromatography system (Waters Corp., Milford, MA, USA) was utilized. System control and all of the mass spectrometry data were acquired and analyzed using MassLynx 3.5 (Micromass, Manchester, U.K.). For separation, a symmetry shield RP C_{18} column (50 × 2.1 mm, 3.5 μm particle size) was used. The column temperature was held at 30 °C. The injection volume was 10 μL, and the flow rate of the mobile phase was 200 μL/min.

The quantitative determination of EXP3174 for all samples was performed using a Quattro micro TM (Micromass, Waters) mass spectrometer. Data were acquired in the electrospray ionization (ESI) mode with positive ion detection and single ion recording (SIR). A cone voltage of 25 V and capillary voltage of 3.50 kV were used. The desolvation temperature was maintained at 300 °C. Argon was used as the API gas, and nitrogen was used as the collision gas, with a flow rate of 50 and 250 L/h respectively. For samples taken from losartan groups, the concentration of losartan was also simultaneously determined. The *m*/*z* values of the parent and daughter ions were set to 437.1 and 207.1 for EXP3174, 423.1 and 207.1 for losartan, and 441.1 and 263.1 for candesartan, respectively. The duration of analysis was 4 min in this assay method.

Pharmacokinetic Data Analysis and Statistical Analysis. The plasma concentration−time data of losartan or EXP3174 were fitted using the WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA) computer program, and the pharmacokinetic parameters were obtained. The area under the concentration−time curve (AUC_{0-24h}) was determined using the trapezoidal method. C_{max} and T_{max} values were determined through the observation of individual animal concentration versus time curves. All mean values obtained in this work are presented with their standard deviation (mean ± SD). The pharmacokinetic parameters were compared using a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett's correction. Differences were considered to be significant at a level of $p < 0.05$.

Results and Discussion

Aqueous Solubility and pH Stability. The aqueous solubility of EXP3174-pivoxil and losartan was determined

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after a 24 h equilibrium period. The solubility of EXP3174-pivoxil was as low as 0.7 ± 0.1 μg/mL at pH 1.2. The solubility values for losartan at pH 6.8 and water were 10.4 ± 1.3 μg and 71.4 ± 5.8 μg/mL, respectively. Moreover, those for EXP3174-pivoxil at pH 6.8 and water were 9.8 ± 0.8 μg and 84.4 ± 2.9 μg/mL, respectively. As the substitute group changed from the hydroxyl group to the pivaloyloxymethyl ester group, the solubility of EXP3174-pivoxil in the buffer did not decrease and the hydrophilicity of EXP3174-pivoxil remained almost at the same level, compared with losartan. Both the solubilities of EXP3174-pivoxil and losartan were enhanced by increasing the pH value of the solvent, which could be explained by the intrinsic weak acidity of the drugs.

The influence of pH on the overall rate of hydrolysis of EXP3174-pivoxil was investigated at 37 °C. The purity of EXP3174-pivoxil varied within a very narrow range and did not substantially change in any buffer system during the testing period. The ester prodrug did not suffer from any appreciable degradation, and a tendency for increased susceptibility to hydrolysis was not observed as the pH increased from the acid range toward the alkaline range. Thus, EXP3174-pivoxil was not subjected to any acid- or base-specific catalyzed hydrolysis. The ester prodrug exhibited predictable stability in the pH range of 1.2−9.0.

Powder Stability of EXP3174-Pivoxil. In this study, the physical and chemical stability of EXP3174-pivoxil powder was evaluated. The stability of the EXP3174-pivoxil powder, which was kept in a glass vial with or without a rubber stopper, was evaluated under accelerated and stressed conditions. The water content of EXP3174-pivoxil in closed vials was not significantly different from the initial values. It remained at 0.52−0.62% throughout the storage period. However, the water content % of EXP3174-pivoxil in open vials increased as the relative humidity increased from 60% to 75%, especially at 75% RH. It increased to more than 1% after 1 day of storage and remained at a high level (but lower than 1.2%) during subsequent storage. However, according to the purity test, no obvious degraded compounds were formed, judging by the area (%) of the EXP3174-pivoxil, which was consistently at a level of 98.6%. No loss of drug content was observed under any conditions, despite the increase in water content. Unobservable hydrolysis in the powder state suggested the high stability of the EXP3174-pivoxil.

Metabolism in Liver and Intestinal S9 Fraction in Vitro. The rate of metabolism of EXP3174-pivoxil was determined in liver S9 fractions from rat, dog, and human (as shown in Figure 3A−C), respectively. The ester prodrug was readily hydrolyzed to EXP3174 in all species within 5 min. EXP3174-pivoxil was also found to disappear completely within 30 min and to be transformed into EXP3174 in intestinal S9 in all three species with a half-life of 0.28, 0.27 and 0.25 h for human, dog and rat, respectively (Table 1). These results suggested efficient conversion of EXP3174-pivoxil into EXP3174 in the liver and intestinal fractions from all species, with a higher hydrolysis rate in the former fraction.

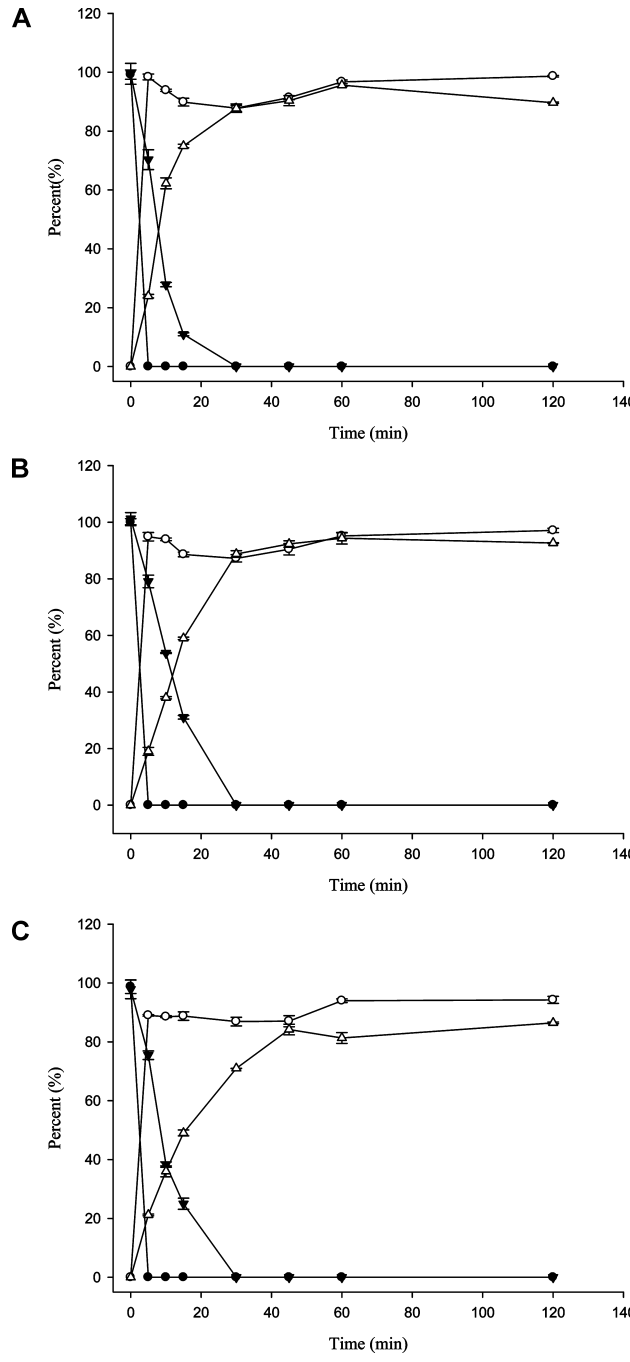

Figure 3. Percentage of remaining EXP3174-pivoxil and formed metabolite (EXP3174) in liver and intestinal S9 fraction from rat (A), dog (B) and human (C): (●) remaining EXP3174-pivoxil in liver S9 fraction, (○) EXP3174 formed in liver S9 fraction, (▼) remaining EXP3174-pivoxil in intestinal S9 fraction, (△) EXP3174 formed in liver S9 fraction. Each point represents the mean ± SD ($n = 3$).

However, for losartan, there was no obvious metabolism in either the liver or intestinal S9 fractions based on the high

Table 1. Stability Half-Life of EXP3174-Pivoxil and Losartan in Liver and Intestinal S9 Fractions from Rat, Dog and Human[a]

	stability half-life ($t_{1/2}$) (h)					
	liver S9			intestinal S9		
compd	rat	dog	human	rat	dog	human
EXP3174-pivoxil				0.28 ± 0.02	0.27 ± 0.03	0.25 ± 0.03
losartan	41.75 ± 3.56	57.75 ± 6.63	96.25 ± 5.23	47.47 ± 2.36	57.27 ± 2.21	113.61 ± 5.37

[a] Each value represents the mean ± SD ($n = 3$).

remaining percentages (over 92%). No EXP3174 was detected until 120 min. Losartan had a significantly longer stable half-life both in the liver and intestinal S9 fractions (41.75 and 47.47, 57.75 and 57.27, 96.25 and 113.61 h for human, dog and rat, respectively) compared with those of the ester prodrug (Table 1). Thus, relatively more rapid biotransformation of ester prodrug into EXP3174 in vivo might be predicted in comparison to losartan.

Regional Intestinal Absorption. The mean plasma concentration−time profiles following administration of EXP3174-pivoxil and losartan into the small intestine (duodenum, jejunum and ileum) are shown in Figure 4A−C. Administration of EXP3174-pivoxil into the jejunum resulted in values for the AUC_{0-24h}, C_{max}, T_{max}, and half-life ($t_{1/2}$) of EXP3174 that were similar to the values resulting from duodenum administration (Table 2). The AUC_{0-24h}, C_{max} and $t_{1/2}$ of EXP3174 following ileal dosing were lower ($p < 0.05$) compared to the duodenal and jejunal dosing, but T_{max} remained unchanged. Our results suggested that the absorption of EXP3174-pivoxil mainly occurs in the upper intestine, becoming erratic in the distal small intestine.

Comparison of the mean AUC_{0-24h}, C_{max}, and T_{max} of EXP3174 between losartan and EXP3174-pivoxil group revealed that significantly more complete and faster absorption of EXP3174 was obtained by the EXP3174-pivoxil (Tables 2 and 3). The C_{max} values of EXP3174 from administration via the duodenum, jejunum, and ileum in the EXP3174-pivoxil group were 7.41-, 8.95- and 11.28-fold higher than the corresponding ones in the losartan group, respectively. Accordingly, the AUC_{0-24h} values of EXP3174 from administration via the duodenum, jejunum and ileum were increased by 3.35, 2.59 and 2.69 times, respectively. T_{max} values decreased from 2.4 h (duodenum), 2.6 h (jejunum) and 4.0 h (ileum) in the losartan group to 0.5 h (all three) in the EXP3174-pivoxil group, indicating efficient biotransformation of EXP3174-pivoxil into EXP3174 and fast absorption in rats, this being consistent with the in vitro results mentioned above. Thus, it is hypothesized that the ester prodrug has a rapid onset of action and might reduce the variation in therapeutic response caused by variable metabolite conversion from losartan.

The pharmacokinetic parameters of the parent drug following dosing of losartan in various segments of the intestine are shown in Table 3. Figure 5 presents the corresponding plasma concentration−time curves of losartan obtained from dosing in each section. The AUC_{0-24h}, C_{max}, and T_{max} of losartan obtained from duodenum and jejunum dosing were almost identical. Our results were consistent with previous

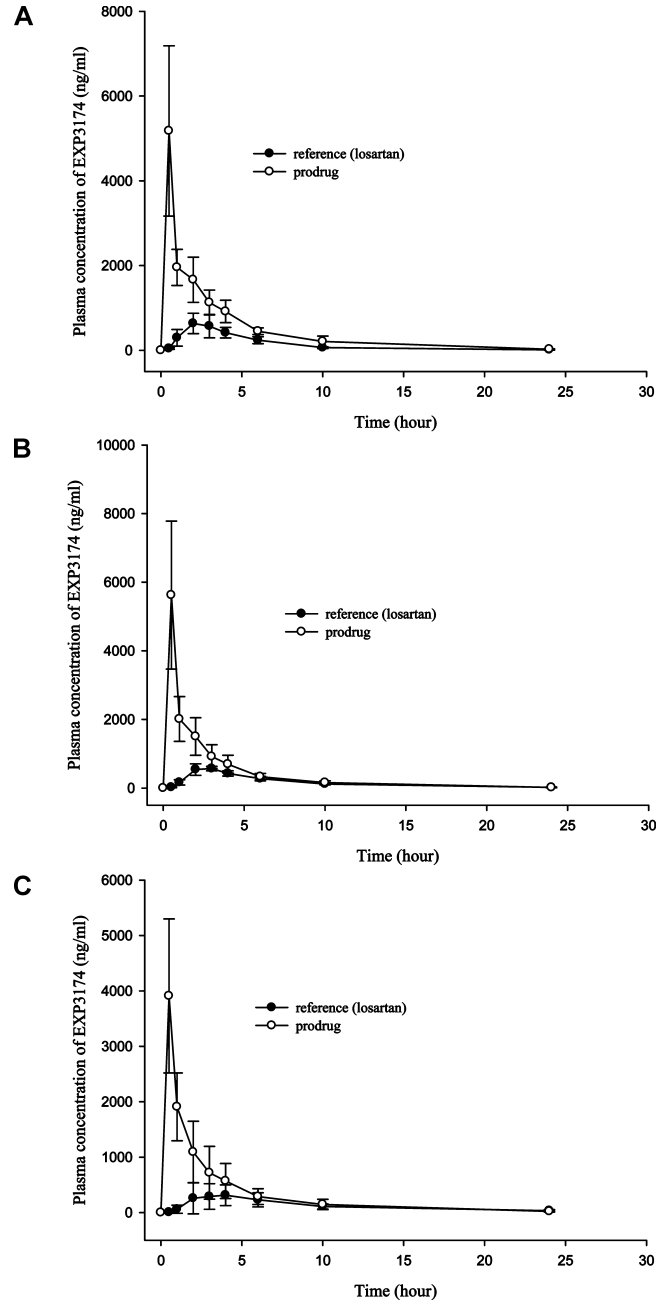

Figure 4. Plasma concentration−time curves of EXP3174 following regional small intestine (duodenum (A), jejunum (B), ileum (C)) administration of losartan (●) and EXP3174-pivoxil (○) (5 mg/kg as EXP3174) to male rats. Each point represents the mean ± SD ($n = 5$).

observations that showed that losartan has similar permeability in these two sections of intestine.[24] Even though ileum

Table 2. Pharmacokinetic Parameters of EXP3174 after Regional Administration of EXP3174-Pivoxil (5 mg/kg, as EXP3174) into the Small Intestine (Duodenum, Jejunum, Ileum) of Male Rats[a]

routes	AUC_{0-24} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
duodenum	11774.1 ± 972.6	5177.0 ± 898.1	0.5 ± 0.0	6.0 ± 0.6
jejunum	10404.3 ± 1408.2	5623.9 ± 965.1	0.5 ± 0.0	6.2 ± 1.1
ileum	8481.9 ± 1711.6	3909.1 ± 621.5	0.5 ± 0.0	5.0 ± 0.4

[a] Each value represents the mean ± SD ($n = 5$).

Table 3. Pharmacokinetic Parameters of Losartan and EXP3174 after Regional Administration of Losartan (5 mg/kg, as EXP3174) into the Small Intestine (Duodenum, Jejunum, Ileum) of Male Rats[a]

routes	AUC_{0-24} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
		Lorsartan		
duodenum	3239.9 ± 119.6	796.3 ± 116.4	1.0 ± 0.3	6.2 ± 2.9
jejunum	3235.0 ± 598.5	823.4 ± 116.1	1.2 ± 0.3	8.1 ± 2.6
ileum	4780.5 ± 516.0	477.6 ± 83.2	1.9 ± 0.7	9.5 ± 3.0
		EXP3174		
duodenum	3512.4 ± 486.6	698.5 ± 111.2	2.4 ± 0.2	5.3 ± 0.7
jejunum	4019.0 ± 281.3	628.7 ± 50.0	2.6 ± 0.2	8.1 ± 0.6
ileum	3157.5 ± 799.1	346.6 ± 109.0	4.0 ± 0.6	9.4 ± 2.1

[a] Each value represents the mean ± SD ($n = 5$).

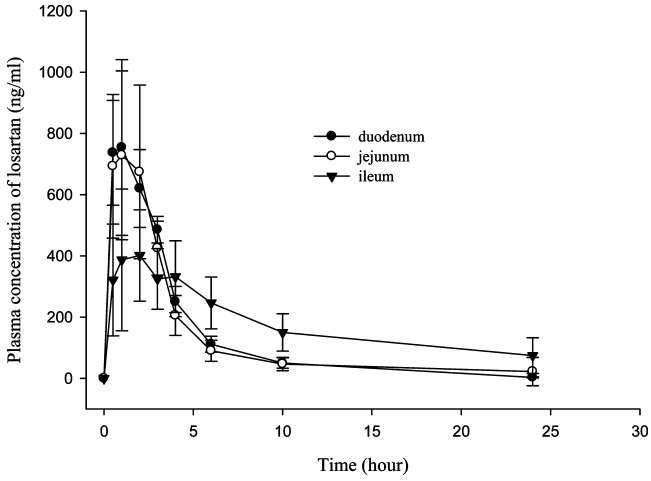

Figure 5. Plasma concentration−time curves of losartan following regional small intestine (duodenum (●), jejunum (○), ileum (▼)) administration of losartan (5 mg/kg as EXP3174) to male rats. Each point represents the mean ± SD ($n = 5$).

administration gave a lower C_{max} compared with the other two groups, it gave the highest AUC_{0-24h} of losartan. The significance of this finding requires further investigation. Interestingly, all the C_{max} values of losartan from duodenum, jejunum and ileum administration were slightly higher than the respective values for EXP3174. Relatively longer T_{max} values for the active metabolite might reflect the fact that significant oxidation of losartan occurred mainly in the liver rather than the intestine.[25]

Table 4. Pharmacokinetic Parameters of EXP3174 after a Single Oral Administration of EXP3174-Pivoxil to Male Rats[a]

dose (mg/kg)	AUC_{0-24} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
0.5	3075.8 ± 432.6	1043.8 ± 337.5	0.5 ± 0.0	5.4 ± 0.2
1.0	7818.2 ± 1017.1	2784.4 ± 374.8	0.5 ± 0.0	5.1 ± 0.2
2.5	16790.2 ± 4160.0	6572.7 ± 2085.2	0.5 ± 0.0	10.6 ± 0.5
5.0	37452.0 ± 4882.2	12929.0 ± 2715.5	0.6 ± 0.1	10.2 ± 0.8

[a] Each value represents the mean ± SD ($n = 5$).

Pharmacokinetic Properties of EXP3174-Pivoxil. The results of analysis of the levels of EXP3174 following oral administration of the EXP3174-pivoxil at various doses are summarized in Table 4. The C_{max} values increased by 2.67, 6.30 and 12.40 times as the dose increased from 0.5 to 1 mg/kg, 0.5 to 2.5 mg/kg and 0.5 to 5 mg/kg, respectively. The corresponding AUC_{0-24h} values were increased by 2.54, 5.45 and 12.18 times, respectively. A statistically significant dose effect was apparent in the AUC_{0-24h} and C_{max}. The values of AUC_{0-24h} and C_{max} increased in proportion to the dose increase within the range of the present study (Figure 6A,B). The relationship between values of AUC_{0-24h} and C_{max}, and the dose for EXP3174, could be expressed by a regression equation with a correlation coefficient r value over 0.9990. The $t_{1/2}$ values at low dose levels (0.5 and 1 mg/kg) were about 5 h. However, those values at the high dose levels (2.5 and 5 mg/kg) were significantly increased to 10.6 and 10.2 h, respectively. The difference in $t_{1/2}$ may have resulted from the longer retention time and subsequent longer absorption process of the ester prodrug in vivo, caused by increased dose-loading.

The comparative profiles of EXP3174 and losartan concentrations in plasma versus time following oral administration of EXP3174-pivoxil at a dose of 1 mg/kg and losartan at a dose of 5 mg/kg are shown in Figure 7. The pharmacokinetic parameters for EXP3174-pivoxil and losartan in plasma for these two groups are presented in Tables 4 and 5. The C_{max} values of EXP3174 in plasma were 2787.4 and 1625.5 ng/mL for the EXP3174-pivoxil and losartan groups, respectively. The C_{max} value was significantly higher for EXP3174-pivoxil than for losartan at this dose level. The AUC_{0-24h} values in plasma were 7818.2 and 7711.9 ng·h/mL, respectively. Although the losartan dose was 5-fold that of EXP3174-pivoxil, there was no significant difference in the AUC_{0-24h} of EXP3174 between these two groups. The T_{max} values of EXP3174 were 0.5 and 2.8 h for EXP3174-pivoxil and losartan, respectively. The latter was significantly longer than that of EXP3174-pivoxil. This difference in T_{max} may be due to the difference in the conversion rate of

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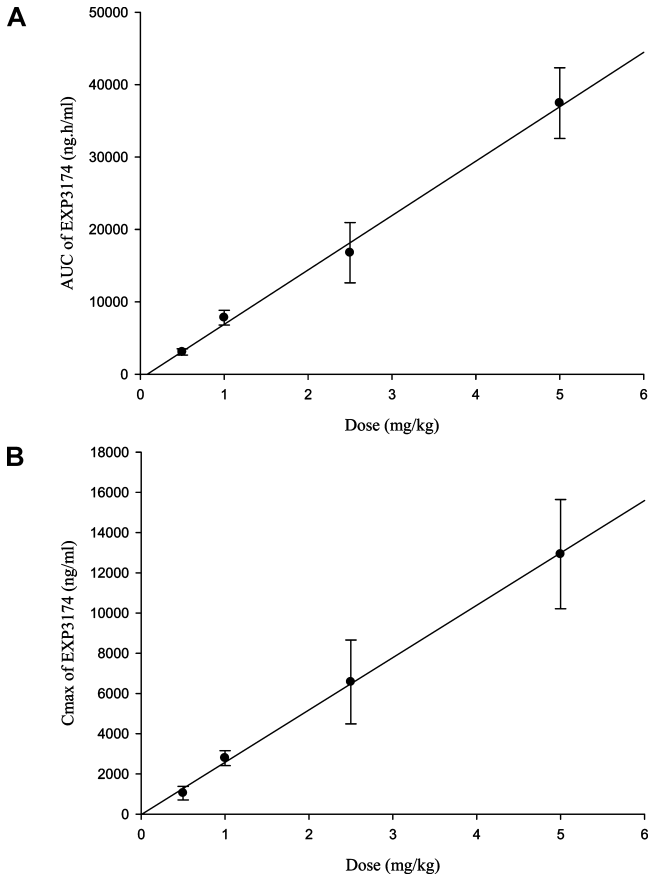

Figure 6. The dose-dependent AUC_{0-24h} (A) and Cmax (B) of EXP3174 following a single oral administration of EXP3174-pivoxil to male rats (0.5, 1.0, 2.5, 5 mg/kg as EXP3174, respectively, po). Each point represents the mean ± SD ($n = 5$).

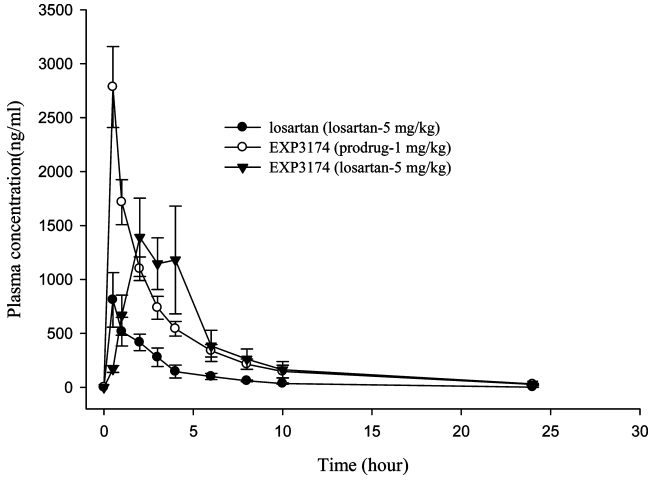

Figure 7. Mean plasma concentration−time profiles of EXP3174 following a single oral administration of EXP3174-pivoxil (○) and losartan (▼) (1.0 and 5.0 mg/kg as EXP3174, respectively) and the mean plasma concentration−time profile of losartan following a single oral dose of losartan (●) (5.0 mg/kg as EXP3174). Each point represents the mean ± SD ($n = 5$).

EXP3174 from the parent drug in the gastrointestinal tract and possibly in the liver microsome.

Table 5. Pharmacokinetic Parameters of Losartan and EXP3174 after a Single Oral Administration of Losartan (5 mg/kg as EXP3174) to Male Rats[a]

	AUC_{0-24} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
losartan	2330.8 ± 432.5	810.3 ± 253.0	0.5 ± 0.0	3.8 ± 0.6
EXP3174	7711.9 ± 1578.6	1626.5 ± 231.5	2.8 ± 0.4	5.5 ± 0.2

[a] Each value represents the mean ± SD ($n = 5$).

The relationship between the plasma concentrations of EXP3174 and losartan following oral administration of losartan in rats was investigated. As shown in Table 5, the C_{max} and AUC_{0-24h} values of EXP3174 were about 2.0- and 3.3-fold higher than those of losartan after a single dose of losartan, respectively. Furthermore, the concentrations at most time points for EXP3174 were higher than those of losartan (see Figure 7). These findings are consistent with previously reported results.[26] In contrast to losartan, EXP3174 also showed a longer T_{max} and $t_{1/2}$.

Even though EXP3174 is more potent than losartan as an antihypertensive agent, its low bioavailability limits its clinical efficacy and application,[4] Thus, no product using EXP3174 as an active substance is commercially available to date. However, both EXP3174-pivoxil and a marketed product of losartan have the common active metabolite of EXP3174, which is mainly responsible for their pharmacological effects in vivo after oral administration. Accordingly, losartan was employed as a reference drug in in vivo absorption studies, and the plasma concentrations and bioavailability of EXP3174 obtained from both compounds were compared to assess the clinical potency of the synthesized prodrug. A similar investigational method has already been applied in the comparative evaluation of two prodrugs of zidovudine.[27]

The pharmacokinetic study revealed a 5-fold enhancement in bioavailability of EXP3174, and even higher plasma concentrations of EXP3174 are obtained when EXP3174-pivoxil is applied. Since the correlation between the plasma concentration of EXP3174 and its pharmacodynamic effects has been well demonstrated both in human and animal models,[28,29] this observation suggests the opportunity to apply lower doses using EXP3174-pivoxil than usually recommended for losartan. Thus, some undesirable gas-

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trointestinal disturbances such as nausea and vomiting that accompany the oral administraton of losartan might be avoided. Additionally, another risk of carnitine depletion invoked by the release of pivalic acid from the breakdown of the EXP3174-pivoxil in vivo can also be minimized by taking advantage of the lower effective dose. However, further studies are needed to verify these hypotheses.

Conclusions

In conclusion, the EXP3174-pivoxil ester prodrug had good pH stability in aqueous solution as well as powder stability. The in vitro metabolism study demonstrated that the ester prodrug was rapidly and sufficiently converted into EXP3174 in both the liver and intestinal S9 fractions from rat, dog and human with a higher rate of hydrolysis in the former fraction. The in vivo absorption study by regional intestinal dosing revealed that duodenum and jejunum administration resulted in a higher AUC_{0-24h} and C_{max} than those following ileal dosing. The absorption of EXP3174-pivoxil from all three segments was faster than that of losartan and EXP3174-pivoxil was more efficiently biotransformed into EXP3174 than that of losartan in rats. This observation made it possible to predict that a more rapid and constant therapeutic response could be achieved by ester prodrug compared to that of losartan, which showed great variation in the rate of biotransformation from the parent drug to EXP3174. The pharmacokinetics studies performed in the rats revealed that the values of AUC_{0-24h} and C_{max} of EXP3174 increased proportionally as the dose escalated from 0.5 mg to 5 mg/kg. The shorter T_{max} might reflect the faster conversion of the parent drug into the active metabolite in vivo compared to that of losartan. The oral bioavailability was significantly enhanced by 5-fold by ester prodrug compared to that of losartan. Thus, based on the combined results, our results suggest that the novel synthesized EXP3174-pivoxil is an ideal candidate with high clinical potential, since it could be applied clinically at a lower dose than that of losartan, and it also showed a more rapid and consistent therapeutic response compared to that of losartan. However, this theoretical hypothesis needs further verification.

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