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Enhanced bioavailability of piroxicam via salt formation with ethanolamines

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Abstract

Piroxicam can be ionized as a zwitterion that has two pKa values ($pKa_1 = 1.86$ and $pKa_2 = 5.46$). Consequently, piroxicam has a low solubility in both polar and nonpolar media, and a low lipophilicity, which results in a low permeability. Three piroxicam-ethanolamine salts were prepared, which had a higher area under the curve (AUC) than piroxicam. There were minimal differences in the AUC among the salt forms. It was reported that the piroxicam triethanolamine salt had a lower permeability across the skin than piroxicam but it had a higher oral bioavailability. Piroxicam monoethanolamine showed the highest C_{max} followed by piroxicam diethanolamine and piroxicam triethanolamine. The dissolution rates of piroxicam and its salts were similar at pH 1.2. Piroxicam monoethanolamine showed the highest dissolution rate at pH 6.8, which was followed by the piroxicam diethanolamine and piroxicam triethanolamine salts. The order of dissolution rate at pH 6.8 matched the order of C_{max} or the AUC after oral administration.
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Keywords: Piroxicam; Ethanolamine; Salt; Dissolution; Bioavailability

1. Introduction

Piroxicam is one of the most potent non-steroidal anti-inflammatory drugs. It is an ionizable water-insoluble drug at a physiological pH. Specifically, piroxicam can be ionized as a zwitterion with two pKa values (Fig. 1, $pKa_1 = 1.86$ and $pKa_2 = 5.46$) (Jinno et al., 2000). A zwitterionic drug possesses a large intramolecular multipole moment due to its multiplicity of oppositely charged groups. Consequently, most of these drugs have a low solubility in polar and nonpolar media, as well as a low lipophilicity. Although piroxicam belongs to class 2 with a low solubility and high permeability based on the Biopharmaceutics Classification System (Lipka and Amidon, 1999), a pharmacokinetic study of piroxicam revealed that it takes

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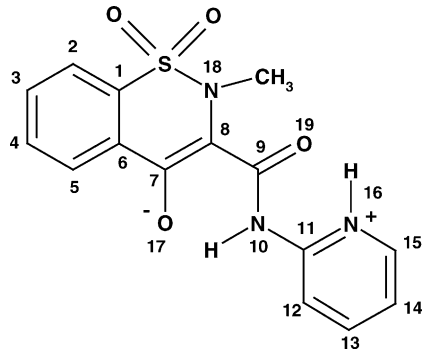

Fig. 1. The structure of the zwitterion form of piroxicam.

more than 2 h to reach the maximum concentration, indicating the slow absorption rate after being administered orally (Tagliati et al., 1999). The bioavailability of a drug with a low solubility can be improved by increasing its solubility. Numerous techniques have been used to increase the solubility of poorly water soluble drugs (Yüksel et al., 2003). Piroxicam has been used as an analgesic where the rapid onset of reaction is desirable. In order to enhance the solubility of piroxicam and to improve its bioavailability, piroxicam was dissolved in lipophilic vehicle (4). A zinc complex of piroxicam reduced the level of gastric irritation. However, it did not improve its bioavailability. A complex of piroxicam with cyclodextrin increased the area under the curve (AUC) but the maximum concentration was not changed significantly (Kimura et al., 1997). A solid dispersion of piroxicam using PEG 400 showed an improved dissolution rate (Fernández et al., 1993).

It was reported that the formation of a salt with monoethanolamine or diethanolamine improved the percutaneous absorption of piroxicam (Cheong and Choi, 2002). Therefore, this study examined the effect of the ethanolamine salts of piroxicam on the pharmacokinetics of piroxicam after oral administration and compared these results with the results obtained from a percutaneous absorption study.

2. Materials and methods

2.1. Materials

Piroxicam and tenoxicam were a kind gift from Jeil Pharm. (Seoul, South Korea). The monoethanolamine, diethanolamine, and triethanolamine were purchased from Sigma Chemical (St. Louis, MO). The ketamine hydrochloride was obtained from Yuhan Corp. (Seoul, South Korea). The polyethylene tube (0.58 mm i.d. × 0.96 mm o.d.) was purchased from Naume Corp. (Tokyo, Japan). All other chemicals were of reagent grade or above and were used without further purification.

2.2. Methods

2.2.1. Preparation of piroxicam ethanolamine salts

The piroxicam ethanolamine salts were prepared using the method reported in the literature (Cheong and Choi, 2002). Briefly, piroxicam was dissolved in methylene chloride and an equi-molar amount of each ethanolamine was added. The solutions were stirred for 24 h, and the precipitated salts were collected by filtration. The light yellow solid residues [i.e. $(C_2H_8NO)^+(C_{15}H_{12}N_3O_4S)^-$, $(C_4H_{12}NO_2)^+(C_{15}H_{12}N_3O_4S)^-$, $(C_6H_{16}NO_3)^+(C_{15}H_{12}N_3O_4S)^-$] were dried in a vacuum for 3 h. The sum of the weight of piroxicam and ethanolamine added was equal to the weight of the precipitate.

2.2.2. Dissolution

The release rates of piroxicam and its salts were measured using a dissolution tester (DST-810, LABFINE, Inc., Korea). Piroxicam and its salts were filled into gelatin capsule (50 mg as piroxicam). Each capsule was placed in 900 ml of a dissolution medium and stirred at 50 rpm at 37 °C. The pH values of the dissolution medium tested were 1.2 and 6.8. An aliquot of the release medium (5 ml) was withdrawn at predetermined time intervals, and an equivalent amount of fresh medium was added to the dissolution medium. The collected samples were filtered through a 0.45-μm syringe filter and analyzed by HPLC (Shimadzu Scientific Instruments, MD, USA), which consisted of a UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A). The wavelength of the UV detector was set at 320 nm and a reversed phase column (Alltima C8, Alltech associates, IL) was used. The column temperature was maintained at 30 °C using a thin foil temperature controller (CH 1445, SYSTEC, MN). The flow rate was 1 ml/min and methanol/water/phosphoric acid (700/299/1) was used as the mobile phase. The

piroxicam salts would be dissociated into piroxicam and ethanolamines under our analytical conditions of pH 3.0. Therefore, we analyzed the salt samples as piroxicam. The retention time of piroxicam was 3.3 min under our experimental conditions.

2.2.3. Pharmacokinetic studies of piroxicam salts

Male Wistar rats weighing 280–320 g were obtained from Samtako Bio Co., Ltd. (Osan, Korea). The rats were anesthetized with 1 ml/kg of ketamine hydrochloride (50 mg/ml) and the right femoral artery was cannulated using polyethylene tubes (0.58 mm i.d. × 0.96 mm o.d.). After surgery, each animal was housed individually in a cage. The animals were fasted overnight until the end of the experiment but were allowed water ad libitum. The rats were then divided into four groups, comprising six rats each. Groups 1–4 were administered piroxicam (PX), piroxicam monoethanolamine salt (PX-MEA), piroxicam diethanolamine salt (PX-DEA), and piroxicam triethanolamine salt (PX-TEA), respectively. A dose equivalent to 10 mg/kg of piroxicam dispersed in 1 ml of water was administered orally to each of the animals. All the studies were carried out according to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences.

A plasma sample (0.15 ml) was collected from the artery cannula before and 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 h after administering the drug. The catheter was flushed with 0.15 ml of heparin (100 U/ml) following each blood sample collection. All samples were stored at −70 °C until analyzed.

2.2.4. Piroxicam assay

The piroxicam sample was assayed using a high-performance liquid chromatography (HPLC) system. 0.15 ml of tenoxicam (250 μg/ml), which was used as an internal standard (IS), was added to 0.1 ml of the plasma samples. The samples were then acidified by adding 0.2 ml of 0.1N hydrochloride and extracted with 7 ml of diethyl ether for 3 min using a vortex mixer. The tubes were centrifuged at 3000 rpm for 10 min. The organic layer was pooled in a conical borosilicate centrifuge tube, and back-extracted with 0.2 ml of 0.02N sodium hydroxide by vortex-mixing for 3 min. After centrifuging for 10 min at 3000 rpm, 20 μl of the aqueous layer was injected into the HPLC system.

The chromatographic system consisted of a pump (LC-10AD), an automatic injector (SIL-10A) and a UV detector (SPD-10A) (Shimadzu Scientific Instruments, Japan) set at 361 nm. An ODS column (μBondapak C18, 3.9 mm × 300 mm, 10 μm, Waters, USA) was eluted with a mixture of a 10 mM phosphate buffer (pH 2) and MeOH (45:55 v/v) at a flow rate of 1 ml/min and at room temperature. A calibration curve was constructed based on the peak area measurements. The limit of quantitation (LOQ) of piroxicam was determined as the sample concentration of piroxicam resulting in peak heights of 10 times baseline noise. The LOQ was found to be 1 μg/ml. Based on three times peak height of baseline noise, the limit of detection was calculated to be 0.1 μg/ml. The intra- and inter-day precisions of the methods were determined by the assay of five samples of drug-free plasma containing known concentrations of piroxicam. The intra- and inter-day percentage of relative standard deviation (R.S.D.) (%) at the concentrations above LOQ was within 9% (15.7% at LOQ), which were acceptable for all quality control samples including the LOQ. The accuracy of piroxicam ranged between 91.5 and 111.3%.

2.2.5. Analysis of results

Pharmacokinetic analysis was performed using compartmental and non-compartmental methods in WinNonlin (Version 1.1, Scientific Consulting Inc., Cary, NC, USA). The piroxicam concentration–time curves were fitted to a one-compartment model with a first order absorption. The area under the plasma concentration–time profile (AUC_{0-t}) was calculated using the log-linear trapezoidal method. The elimination half-life ($t_{1/2}$) was estimated from the slope of the terminal phase of the log plasma concentration–time points fitted using the least-squares method. The additional estimated parameters were the systemic plasma clearance (Cl/F/kg) and the volume of distribution (Vz (terminal)/F/kg). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were determined directly from the individual blood concentration against the time curves.

2.2.6. Statistical analysis

All the values are expressed as a mean ± S.E. The pharmacokinetic variables from the four dosage forms were compared with a one-way ANOVA, which followed by a posterior testing with an unpaired *t*-test us-

ing the Bonferroni correction. A *P*-value of less than 0.05 was considered significant.

3. Results

Fig. 2 shows the mean plasma concentration–time profiles after the oral administration of PX, PX-MEA, PX-DEA, and PX-TEA to the rats at a dose of 10 mg/kg ($n = 6$). Compared to PX alone, the salt forms tend to show higher plasma concentrations. The plasma concentrations decreased in the order of PX-MEA > PX-DEA > PX-TEA > PX (statistically significant difference between PX and PX-MEA). It was reported that PX-MEA showed the highest percutaneous absorption rate across the hairless mouse skin followed by PX-DEA (Cheong and Choi, 2002). One different aspect of this study from the percutaneous absorption study is that although there was no statistically significant difference, the TEA salt formation of PX showed higher plasma profile of PX after the oral administration, while PX-TEA showed a slightly lower permeation rate than PX after topical application. The higher percutaneous permeation rate of PX-MEA and PX-DEA were attributed to the lower crystalline lattice energy and the higher solubility in both polar and non-polar solvents. Based on the fact that PX belongs to the class 2 compounds with a high permeability and a low solubility,

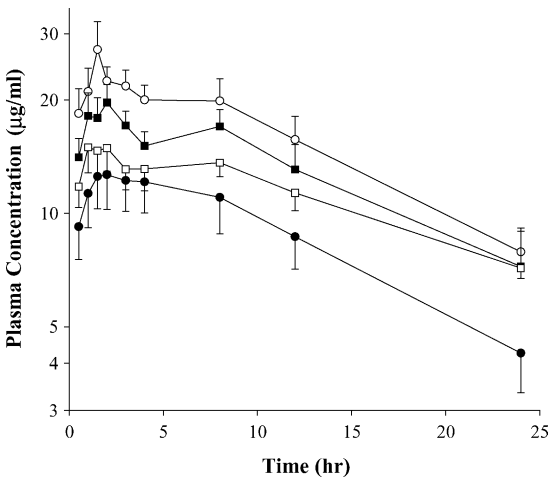

Fig. 2. Plasma concentration profiles of piroxicam after the oral administration of: (●) PX, (◯) PX-MEA, (■) PX-DEA, and (□) PX-TEA. The data is expressed as a mean ± S.E. ($n = 6$).

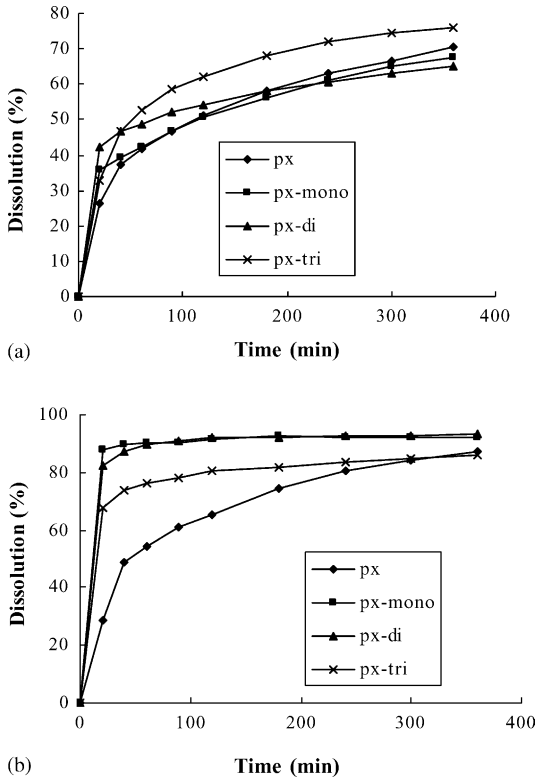

Fig. 3. Dissolution profiles of PX, PX-MEA, PX-DEA, PX-TEA at pH 1.2 (a) and pH 6.8 (b). px: PX, px-mono: PX-MEA, px-di: PX-DEA, px-tri: PX-TEA.

it is likely that an increase in the dissolution rate would result in a higher bioavailability because the dissolution rate is the rate controlling step in drug absorption following the oral administration of class 2 compounds.

In order to determine the relationship between the dissolution rate and the bioavailability, the dissolution profiles of PX and its salts were measured and the results are shown in Fig. 3. With the exception that the dissolution rate of PX-TEA showed a slightly higher dissolution rate in the later phase of the dissolution study, the dissolution rates of PX and its salts were similar at pH 1.2. The dissolution rates of PX and its salts at pH 1.2 were slower than those at pH 6.8. Based on small volume of gastric fluid and short residence time in the stomach, this indicates that PX and its salts will be dissolved and absorbed mainly in the intestine. At pH 6.8, the dissolution rate of PX-MEA was the highest followed by PX-DEA, PX-TEA, and PX. The order of the dissolution rate at pH 6.8

Table 1
Pharmacokinetic parameters of piroxicam (PX), piroxicam monoethanolamine (PX-MEA), piroxicam diethanolamine (PX-DEA), and piroxicam triethanolamine (PX-TEA) after the oral administration to rats

	PX	PX-MEA	PX-DEA	PX-TEA
C_{max} (μg/ml)	13.6 ± 2.1	29.2 ± 4.7[a,b]	22.1 ± 1.9	16.1 ± 1.7
t_{max} (h)	2.2 ± 0.3	2.9 ± 1.0	3.6 ± 1.4	3.6 ± 1.4
$t_{1/2}$ (h)	11.9 ± 1.1[b]	12.9 ± 1.7[b]	13.2 ± 1.2[b]	20.1 ± 1.8
AUC_{0-24} (μg h/ml)	197.7 ± 30.6	369.3 ± 40.0[a]	306.3 ± 34.2	263.6 ± 23.4
$AUC_{0-\infty}$ (μg h/ml)	276.8 ± 48.1	520.1 ± 41.7[a]	477.0 ± 55.2	475.6 ± 41.5
Cl/F/kg (l/h)	0.044 ± 0.01	0.021 ± 0.002[a]	0.026 ± 0.002[a]	0.022 ± 0.002[a]
Vz/F/kg (l)	0.69 ± 0.07	0.38 ± 0.07[a]	0.42 ± 0.04[a]	0.62 ± 0.09

Data were expressed as the mean ± S.E. ($n = 6$).
[a] $P < 0.05$ vs. PX.
[b] $P < 0.05$ vs. PX-TEA.

matched that of the plasma concentration profile. The results of the dissolution study indicate that the higher plasma profile of PX obtained by the formation of ethanolamine salts is mainly due to the increased dissolution rate of the PX salts. It is interesting to note that PX-MEA and PX-DEA dissolved quite rapidly, with most of the drug being released within 20 min and no significant dissolution being observed thereafter. Although the dissolution profile of PX-TEA also showed a faster initial release rate than PX, the extent of dissolution did not reach 80% after 360 min. In contrast, the dissolution of PX gradually increased with time until the end of the dissolution study. The initial dissolution rate of PX-TEA was significantly higher than that of PX. However, the trend was reversed in the later phase and the total amount of PX released was slightly higher than that of PX-TEA at the end of the study.

The pharmacokinetic parameters of PX and the three salt forms evaluated by the compartmental and non-compartmental method are shown in Table 1. It was found that there were some statistically significant differences in the C_{max} ($P < 0.005$), AUC_{0-24} ($P < 0.05$), Cl/F/kg ($P < 0.05$), and Vz/F/kg ($P < 0.05$) between the compounds. The rank order of C_{max} matched that of the release rate from dissolution study at pH 6.8. During the PX-MEA phase, the C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ were significantly higher than those of PX; PX-MEA raised the parameters to 215% (range 108–368%, $P < 0.05$), 186% (range 113–250%, $P < 0.05$), and 188% (range 133–249%, $P < 0.05$) of PX, respectively. The Cl/F/kg values of all salt forms were similar (0.021–0.026 l/h), and significantly smaller than that of PX ($P < 0.05$). However, only two salt forms, PX-MEA and PX-DEA, had smaller Vz/F/kg values compared with PX ($P < 0.05$). The significantly prolonged $t_{1/2}$ of PX-TEA compared with those of PX, PX-MEA, and PX-DEA (1.69-fold, 1.56-fold, and 1.52-fold, respectively) can be explained by the decreased Cl/F/kg without decreasing the Vz/F/kg. The mean t_{max}, $t_{1/2}$, Cl/F/kg, and Vz/F/kg of PX were 2.2 h, 11.9 h, 0.044 l/h and 0.69 l, respectively, which were similar to those reported in other studies. (Roskos and Boudinot, 1990; Boundinot et al., 1993; Heeb et al., 2003).

In conclusion, the results demonstrated that the monoethanolamine salt form of piroxicam may be used to shorten the onset of reaction and to improve the bioavailability of piroxicam.

References

Boundinot, S.G., Funderburg, E.D., Boundinot, F.D., 1993. Effects of age on the pharmacokinetics of piroxicam in rats. J. Pharm. Sci. 82, 254–257.

Cheong, H.-A., Choi, H.-K., 2002. Enhanced percutaneous absorption of piroxicam via salt formation with ethanolamines. Pharm. Res. 19, 1372–1377.

Fernández, M., Margarit, M.V., Rodríguez, I.C., Cerezo, A., 1993. Dissolution kinetics of piroxicam in solid dispersions with polyethylene glycol 4000. Int. J. Pharm. 98, 29–35.

Heeb, H.L., Chun, R., Koch, D.E., Goatley, M.A., Hunter, R.P., 2003. Single dose pharmacokinetics of piroxicam in cats. J. Vet. Pharmacol. Ther. 26, 259–263.

Jinno, J., Oh, D.-M., Crison, J.R., Amidon, G.L., 2000. Dissolution of ionizable water-insoluble drugs: the combined effect of pH and surfactant. J. Pharm. Sci. 89, 268–274.

Kimura, E., Bersani-Amado, C.A., Sudo, L.S., Santos, S.R.J., Oga, S., 1997. Pharmacokinetic profile of piroxicam β-cyclodextrin, in rat plasma and lymph. Gen. Pharmacol. 28, 695–698.

Lipka, E., Amidon, G.L., 1999. Setting bioequivalence requirements for drug development based on preclinical data: optimizing oral drug delivery systems. J. Control. Rel. 62, 41–49.

Roskos, L.K., Boudinot, F.D., 1990. Effects of dose and sex on the pharmacokinetics of piroxicam in the rat. Biopharm. Drug Dispos. 11, 215–225.

Tagliati, C.A., Kimura, E., Nothenberg, M.S., Santos, S., Oga, S., 1999. Pharmacokinetic profile and gastric effect of zinc-piroxicam in rats. Gen. Pharmacol. 33, 67–71.

Yüksel, N., Karata, A., Özkan, Y., Savaser, A., Özkan, S.A., Baykara, T., 2003. Enhanced bioavailability of piroxicam using Gelucire 44/14 and Labrasol: in vitro and in vivo evaluation. Eur. J. Pharm. Biopharm. 56, 453–459.