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Enhanced Oral Bioavailability of Piroxicam in Rats by Hyaluronate Microspheres

Ming Guan Piao
College of Pharmacy, Yeungnam University, Dae-Dong, Gyongsan, South Korea

Jeong-Hoon Kim and Jong Oh Kim
Research Lab, Dong-A Pharm. Co. Ltd., Yongin-Si, Kyunggi-Do, South Korea

Won Seok Lyoo
School of Textiles, Yeungnam University, Dae-Dong, Gyongsan, South Korea

Mann Hyung Lee
College of Pharmacy, Catholic University of Daegu, Gyongsan, South Korea

Chul Soon Yong and Han-Gon Choi
College of Pharmacy, Yeungnam University, Dae-Dong, Gyongsan, South Korea

ABSTRACT To enhance the dissolution and oral bioavailability of poorly water soluble piroxicam, the piroxicam-loaded hyaluronic microspheres were prepared with various ratios of piroxicam, sodium hyaluronate and polyethylene glycol 4000 (PEG) using a spray dryer, and their physicochemical properties such as shape, size, drug-loading efficiency and dissolution were investigated. The pharmacokinetic study of piroxicam-loaded hyaluronic micropheres in rats was then performed compared to piroxicam powder. The piroxicam-loaded hyaluronic microspheres, spherical in shape, had the geometric mean diameters of about 1.5 μm and drug loading efficiency of about 90%, irrespective of ratio of piroxicam/sodium hyaluronate/PEG. The hyaluronic microspheres containing PEG gave significantly higher dissolution rates of drug than did piroxicam powder, PEG-based solid dispersion system and hyaluronic microspheres without PEG, suggesting that the hyaluronic microsphere with sodium hyaluronate and PEG was more useful for improving the dissolution rate of poorly water soluble piroxicam. The piroxicam-loaded hyaluronic microcapsule composed of (piroxicam/sodium hyaluronate/PEG; 2: 20: 1) gave about threefold improved dissolution of drug in water for 4 h compared to piroxicam powder. It showed higher plasma concentrations of drug compared to piroxicam powder. It gave significantly higher AUC and faster T_{max} of piroxicam than did piroxicam powder. In particular, the AUC of piroxicam from hyaluronic microsphere was about twofold higher than that from piroxicam powder, suggesting that it could enhance the oral bioavailability of piroxicam. Thus, the hyaluronic microsphere developed using spray-drying technique with sodium hyaluronate and PEG was a more effective oral dosage form for poorly water soluble piroxicam.

KEYWORDS Microspheres, Piroxicam, Sodium hyaluronate, Polyethylene glycol, Dissolution, Bbioavailability

INTRODUCTION

Piroxicam [4-hydroxyl-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide], a nonsteroidal antiinflammatory agent, is widely used in treatment of moderated pain and fever (Sallmann, 1985; Fraser et al., 1997;

Address correspondence to Han-Gon Choi, College of Pharmacy, Yeungnam University, 214-1, Dae-Dong, Gyongsan 712-749, South Korea; E-mail: hangon@yu.ac.kr

Goa & Benfield, 1994). However, it is a drug with low water solubility and high membrane permeability included in class two of the Biopharmaceutical Drug Classification System (Amidon et al., 1995). It is absorbed slowly and gradually through the gastrointestinal tract and consequently the onset of the analgesic and antiinflammatory actions is delayed (Bhattacharyya et al., 1993; Yuksel et al., 2003). Various formulations of poorly water soluble piroxicam such as emulsifying liposphere (Attama & Mpamaugo, 2006), PLGA microsphere (Pollauf et al., 2005), microencapsulation, inclusion complex (Jug & Becirevic-Lacan, 2004; Kimura et al., 1997), HPMC microsphere (Jug et al., 2004) and solid dispersion (Barzegar-Jalali et al., 2002; Bhattacharyya et al., 1993; Pan et al., 2000; Shin & Cho, 1997; Tantishaiyakul et al., 1999; Verma et al., 2003) were developed to improve the bioavailability and solubility of poorly water soluble, lipophilic piroxicam.

In this study, to develop a piroxicam-loaded hyaluronic micropheres for improving the dissolution and oral bioavailability of piroxicam, it was prepared with various ratios of piroxicam, sodium hyaluronate and polyethylene glycol 4000 (PEG) using a spray dryer, and their physicochemical properties such as shape, size, drug-loading efficiency and dissolution were investigated. The pharmacokinetic study of piroxicam-loaded hyaluronic micropheres in rats was then performed compared to piroxicam powder. Hyaluronic acid was used as a drug-loaded carrier of microspheres in this study, because ongoing pharmaceutical and medical research has been concentrating on its use in drug delivery systems (Alkrad et al., 2003; Fraser et al., 1997; Turley & Roth, 1980) in addition to its enhanced dissolution and bioavailability of poorly water soluble drugs (Jederstrom et al., 2004; Jiang et al., 2005; Goa & Benfield, 1994). Hyaluronic acid, a chemically well characterized linear polysaccharide consisting of alternating b (1→4) linked N-acetyl-D-glucosamine and b (1→3) linked D-glucuronic acid was studied (Jouon et al., 1995; Lapcik et al., 1998). This polyanionic polymer has a range of naturally occurring molecular sizes from 1000–10,000,000 Da, and has unique physicochemical properties and distinctive biological functions (Laurent et al., 1995). Furthermore, polyethylene glycol 4000 (PEG) is a nonionic surfactant commonly used in pharmaceutical preparations (Fernández et al., 1993; Yuksel et al., 2003). In this study, like sodium lauryl sulfate, it has been used to prevent hyaluronic microspheres from attaching to the inner wall of spray drying chamber (Kim et al., 1994; Lee et al., 1999) and to enhance the solubility of poorly water soluble drugs in the development of microspheres (Cleland & Jones, 1996; Fernandez-Carballido et al., 2004; Jiang & Schwendeman, 2001; Mallarde et al., 2003).

MATERIALS AND METHODS

Materials

Piroxicam and sodium hyaluronate were from Whawon Pharm. Co. Ltd., Seoul and Shandong Freda Biochem Co., Ltd. (Jinan, China), respectively. Ethanol and polyethylene glycol 4000 (PEG) were obtained from Ducksan Chemical, Seoul and Aldrich Chemical Co. (Milwaukee, WI), respectively. All other chemicals were of reagent grade and used without further purification. Semipermeable membrane tube (Spectra membrane tubing No.1) was purchased from Spectrum Medical Industries Inc. (Los Angeles, CA).

Preparation of Piroxicam-loaded Hyaluronic Microspheres

A Büchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of piroxicam-loaded hyaluronic microsphere. Various amounts of sodium hyaluronate (2–40 g) were dissolved in 50 mL water to obtain aqueous polymer solution, respectively. Two grams of piroxicam was dissolved in 50 mL ethanol to obtain the drug solution. Further, 0–2 g PEG and 50 mL drug solution were then added to 50 mL aqueous polymer solution one after another. The detailed compositions of piroxicam-loaded hyaluronic micropheres are given in Table 1. The resulting clear solutions were delivered to the nozzle at a flow rate of 5 mL/min using a peristaltic pump and thereafter spray-dried at 160°C inlet temperature. The pressure of spray air was 3 kg/cm^2. The flow rate of drying air

TABLE 1 Composition of Piroxicam-loaded Hyaluronic Microspheres

Ingredients (g)	I	II	III	IV	V	VI	VII	VIII
Piroxicam	2	2	2	2	2	2	2	2
Sodium hyaluronate	20	0	2	10	40	20	20	20
PEG 4000	1	1	1	1	1	0	0.2	2

was maintained at the aspirator setting of 10, which indicated the pressure of aspirator filter vessel of –30 mbar. The direction of airflow was the same as that of sprayed products. The diameter of nozzle was 0.7 mm (Kim et al., 1995; Lee et al., 1999).

Determination of Piroxicam Contents in Hyaluronic Microspheres

To make the saturated solution containing the maximum soluble piroxicam contents, excessive amount of microspheres (about 30 mg) were added to 5 mL of 50% ethanolic solution, shaken in water bath for 3 days and filtered through a membrane filter (0.45 μm). The concentration of piroxicam in the resulting solution was then analyzed by high-performance liquid chromatography (HPLC, Jasco UV-975, Japan) equipped with an Inertsil ODS-3 C_{18} column (GL science, 0.5 μm, 15 cm × 0.46 cm i.d.) and UV detector (Model L-7450). The mobile phase consisted of 0.1*M* sodium acetate, acetonitrile and triethanolamine (61:39:0.05, v/v) adjusted to pH 4.0 with glacial acetic acid. The eluent was monitored at 330 nm with a flow rate of 1.5 mL/min (Dadashzadeh et al., 2002; Kimura et al., 1997; Pan et al., 2000; Yuksel et al., 2003).

Shape and Size Distribution of Piroxicam-loaded Hyaluronic Microspheres

The shape and surface of piroxicam-loaded hyaluronic microspheres were examined using a scanning electron microscope (S-4100, Hitachi, Tokyo, Japan). The microspheres were loaded on the specimen stub via double side sticky tape and coated with gold (Hitachi Iron sputter, E-1030) for 5 min at 100–200 mTorr in a shutter coater before taking photograph at an accelerating votage of 15 kV. The size distribution of piroxicam-loaded hyaluronic micropheres was also measured using a light scattering spectrophotometer (Nimcomp 370, Particle Sizing System Inc., Santa Barbara, CA) after adding to water and appropriate dilution (Kim et al., 1994, 1995).

In Vitro Drug Release

In Vitro Experiments

According to USP XXVIII, dissolution test was performed with about 12.5 mg of piroxicam powder and eight formulae of piroxicam-loaded hyaluronic microspheres (equivalent to 12.5 mg piroxicam). Unlike USP method, they were inserted into a semipermeable membrane tube, respectively to prevent nondissolved microsphers or powder from being sampled. Both sides of the tube were then tied up with a thread to prevent leakage and fixed on the paddle. The paddle with semipermeable membrane tube was placed in a dissolution tester (Shinseang Instrument Co., South Korea). Dissolution test was performed at 36.5°C at 100 rpm with 500 mL distilled water as a dissolution medium. At predetermined intervals, 0.5 mL of the medium was sampled and filtered. The filtrate was analyzed by HPLC at the wavelength of 330 nm as described above.

Statistical Analysis

*T*he dissolution data from different formulae were compared for statistical significance by the one-way analysis of variance (ANOVA). The statistical significance of means among different formulations was then compared by multiple range method of least significant difference. All results were expressed as means ± standard deviation (SD).

Pharmacokinetics Study

In Vivo Experiments

Male Wistar rats weighing 250 ± 20 g were fasted for 24–36 hr prior to the experiments but allowed free access to water. Twelve rats were divided into two groups. The rats in each group were administered with piroxicam powder and hyaluronic microsphere [piroxicam/sodium hyaluronate/PEG (2/20/1)] (0.11 g/kg equivalent to 10 mg/kg piroxicam), respectively. All animals care and procedures were conducted according to the Guiding Priciples in the use of animals in toxicology, as adopted in 1989 and revised in 1999 by the Society of Toxicology (SOT, 1999).

Administration and Blood Collection

Each rat, anesthetized in an ether saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube (PE-50, Intramedic®, Clay Adams, Parsippany, NJ) was inserted into the right femoral artery of the rat, all of the incision was covered with wet cotton and the cannula was flushed with 0.2 mL of heparinized

normal saline (80 U/mL) to prevent blood clotting. Piroxicam powder and hyaluronic microspheres were filled in small hard capsule (#9, Suheung capsule Co. Ltd., Seoul, South Korea), and orally administered to rats in each group, respectively. Half mL of blood was collected from the right femoral artery at various intervals and centrifuged at 3000 rpm for 10 min using a centrifuge 5415C (Eppendorf, Seoul, South Korea) (Attama & Mpamaugo, 2006; Bhattacharyya et al., 1993; Tagliati et al.,1999).

Blood Sample Analysis

Plasma (0.18 mL) was mixed with 0.02 mL of methanolic solution containing naproxen (0.5 μg/mL), as an internal standard and 0.02 mL of 60% perchloric acid. It was then centrifuged at 12,000 g for 5 min to precipitate the proteins. Then, the 50 μL of supernatant layer was analyzed by HPLC at the wavelength of 330 nm as described above.

RESULTS AND DISCUSSION

Piroxicam-loaded hyaluronic microspheres were prepared with various ratios of piroxicam/sodium hyaluronate/PEG using a spray dryer. The compositions of the hyaluronic microspheres are illustrated in Table 1. The scanning electron micrograph of the piroxicam-loaded hyaluronic microspheres showed that the major particles were spherical in shape with a smooth surface as shown in Fig. 1. As shown in Fig. 2A, the geometric mean diameters of the piroxicam-loaded hyaluronic microspheres were about 1.5 μm (Kim et al., 1994, 1995). The ratio of piroxicam/sodium hyaluronate/PEG did not significantly affect their particle sizes (Ferna´ndez et al., 1993). Fig. 2B showed that their drug loading efficiency were about

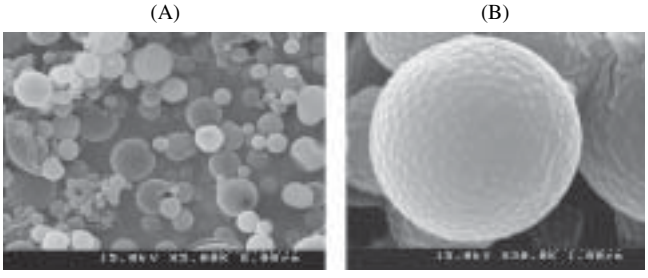

FIGURE 1 **Representative Scanning Electron Micrographs of Piroxicam-loaded Hyaluronic Microspheres: (A) 5,000×; (B) 30,000×.**

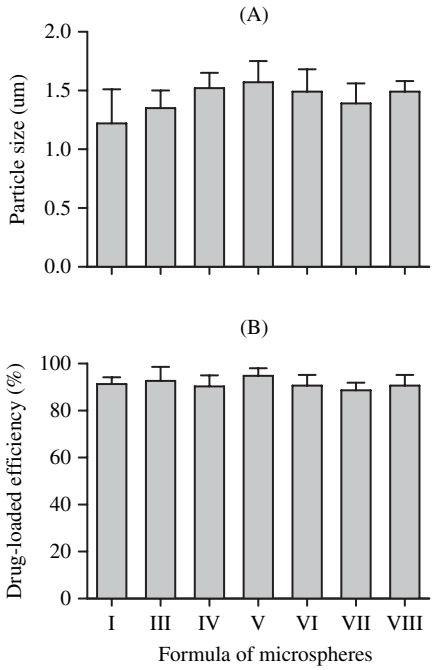

FIGURE 2 **Particle Size and Drug-loading Efficiency of Piroxicam-loaded Hyaluronic Microspheres. Piroxicam-loaded Hyaluronic Microspheres Were Composed of Various Ratios of Piroxicam/Sodium Alginate/PEG 4000. (A) Particle Size; (B) Drug-loading Efficiency.**

90% in all the formulations, regardless of the ratio of piroxicam/sodium hyaluronate/PEG. Moreover, there was no correlation between their particle size and drug-loading efficiency. In this study, we describe the drug-loading efficiency using the following equations (Attama & Mpamaugo, 2006).

$$\text{Drug} - \text{loading efficiency } (\%) = (\text{Cp}/\text{Ct}) \times 100$$

where Cp and Ct are the practical and theoretical drug contents in hyaluronic microspheres, respectively.

To evaluate whether sodium hyaluronate affected the dissolution rates of piroxicam from hyaluronic microsphere, we performed the dissolution test on five formulae of piroxicam-loaded microspheres (Table 1, formula I–V) compared with piroxicam powder. The dissolution profiles of piroxicam from the piroxicam-loaded microspheres are illustrated in Fig. 3. The dissolution rates of drug from four microspheres with sodium hyaluronate (formula I, III–V) significantly increased compared to piroxicam powder. Among four microspheres with sodium hyaluronic acid (formula I, III–V) tested, microsphere with 2 g sodium

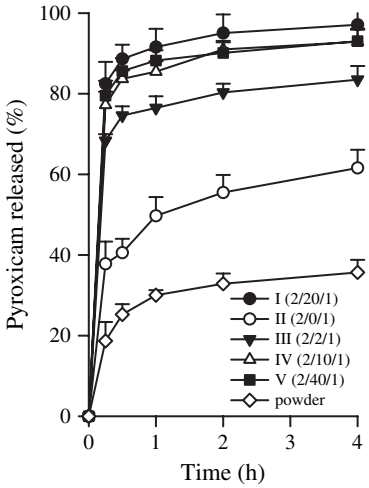

FIGURE 3 **Effect of Sodium Hyaluronate on the Release of Drug From Piroxicam-loaded Hyaluronic Microspheres (Piroxicam/Sodium Alginate/PEG 4000). Each Value Represents the Mean ± SD (*n* = 6).**

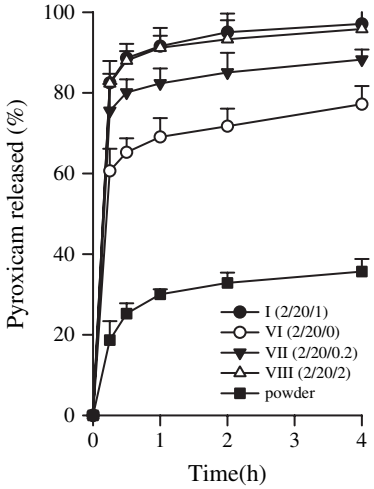

FIGURE 4 **Effect of PEG on the Release of Drug From Piroxicam-loaded Hyaluronic Microspheres (Piroxicam/Sodium Alginate/PEG 4000). Each Value Represents the Mean ± SD (*n* = 6).**

hyaluronate (formula III) gave significantly lowest dissolution rates of drug. However, three microspheres with greater than 10 g sodium hyaluronate (formula I, IV, and V) showed the similar dissolution rates of drug. Our results suggested that the hyaluronic microspheres could improve the dissolution rate of piroxicam in water.

On the other hand, the dissolution rate of piroxicam prepared with PEG alone (formula II) was significantly higher than that of piroxicam powder, indicating that the PEG-based solid dispersion system also improved the dissolution rate of piroxicam. However, the hyaluronic microspheres (formula I, III–V) improved further the dissolution rates of piroxicam compared to PEG-based solid dispersion system without sodium hyaluronate.

To evaluate whether PEG affected the dissolution rates of piroxicam from hyaluronic microsphere, we performed the dissolution studies on four formulae of piroxicam-loaded microspheres (Table 1, formula I, VI–VIII) compared with piroxicam powder. The dissolution profiles of piroxicam from them are illustrated in Fig. 4. The dissolution rates of drug from four hyaluronic microspheres containing PEG (formula I, VII–VIII) significantly increased compared to piroxicam powder. The hyaluronic microspheres containing PEG (formula I, VII, and VIII) showed slightly higher dissolution rates of drug as the amounts of PEG increased. However, there were no significant differences between the dissolution rates of these microspheres containing PEG. Further, the hyaluronic microspheres without PEG (formula VI) gave significantly lower dissolution rates of drug compared to the hyaluronic microspheres containing PEG, while significantly higher dissolution rates of drug compared to piroxicam powder were observed. Our results suggested that the hyaluronic microsphere with sodium hyaluronate and PEG was useful for improving the dissolution rate of poorly water soluble piroxicam (Fernández et al., 1993; Yuksel et al., 2003). The reason for this enhanced dissolution might be dependent upon the enhanced wetting and increased surface area of drug by the simultaneous addition of sodium hyaluronate and PEG.

In particular, the amount of drug dissolved in water for 4 h from the piroxicam-loaded hyaluronic microcapsule composed of [piroxicam/sodium hyaluronate/PEG (2/20/1)] (formula I) increased about threefold compared to piroxicam powder (97.1 ± 3.8 versus 35.7 ± 3.1 %). Thus, this composition was selected as an optimal formula of piroxicam for the oral delivery system in the pharmacokinetics study.

The pharmacokinetic parameters of piroxicam were determined after oral administration of piroxicam powder and the piroxicam-loaded hyaluronic microsphere. The change of mean plasma concentration of piroxicam after oral administration of preparations in rats is shown in Fig. 5. The total plasma concentrations of piroxicam in hyaluronic microsphere were largely higher compared with those in piroxicam powder. In particular, the initial plasma concentrations of piroxicam in hyaluronic microsphere, until 3 h, were significantly higher compared with those in piroxicam powder ($P < 0.05$). Our results suggested that the

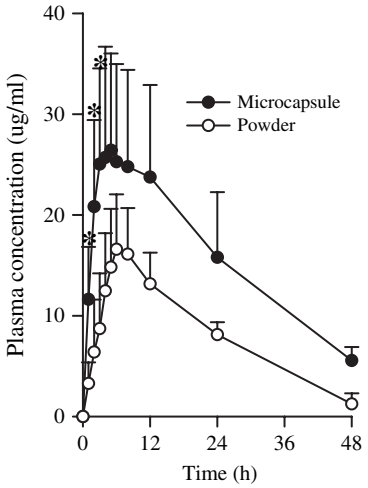

FIGURE 5 **Plasma Concentration-Time Profiles of Piroxicam After Oral Administration of Piroxicam Powder and Piroxicam-loaded Hyaluronic Microspheres to Rats. Piroxicam-loaded Hyaluronic Microspheres Were Composed of (Piroxicam/Sodium Alginate/PEG 4000 [2/20/1]). Each Value Represents the Mean ± SD (n = 6). *p < 0.05 Compared With Powder.**

higher initial plasma concentrations of piroxicam in hyaluronic microspheres were due to the increase in dissolution rate of piroxicam in the hyaluronic microspheres in rats.

The pharmacokinetic parameters are shown in Table 2. The hyaluronic microsphere gave significantly higher AUC and faster T_{max} of piroxicam than did piroxicam powder ($p < 0.05$). The AUC of piroxicam from hyaluronic microsphere was about twofold higher than that from piroxicam powder. The results suggested that the enhanced oral relative bioavailability of piroxicam in the hyaluronic microsphere was contributed by the marked increase in the absorption rate of piroxicam in rats due to the increase in dissolution rate of piroxicam in the hyaluronic microsphere (Dadashzadeh et al., 2002; Kimura et al., 1997; Pan et al., 2000; Yuksel et al., 2003). However, the C_{max}, K_{el}, and $t_{1/2}$ values of piroxicam from hyaluronic microsphere were not significantly different from those compared to piroxicam powder. Thus, the hyaluronic micropheres containing PEG would be useful to deliver piroxicam.

TABLE 2 **Pharmacokinetic Parameters of Piroxicam Delivered by Piroxicam Powder and Piroxicam-loaded Hyaluronic Microspheres**

Parameters	Powder	Microsphere
T_{max} (h)	6.33 ± 0.82	4.33 ± 0.82*
C_{max} (μg/mL)	17.03 ± 4.72	26.99 ± 9.83
AUC (h·μg/mL)	358.31 ± 86.02	763.08 ± 268.41*
$t_{1/2}$ (h)	15.81 ± 3.33	17.44 ± 2.70
K_{el}/h	0.046 ± 0.010	0.041 ± 0.006

*p < 0.05 compared with powder.
**Each value represents the mean ± SD (n = 6).

CONCLUSION

The piroxicam-loaded hyaluronic micropheres developed using a spray-drying technique with sodium hyaluronate and polyethylene glycol 4000 gave threefold higher dissolution and twofold higher AUC of piroxicam compared with piroxicam powder, indicating that the drug from hyaluronic microspheres could be more orally absorbed in rats, leading to a more effective oral dosage form for poorly water soluble piroxicam. The further study on the oral bioavailability in human subjects of piroxicam-loaded preparation will be performed.

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