Preparation and in Vivo Evaluation of Piroxicam-Loaded Gelatin Microcapsule by Spray Drying Technique

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To develop a piroxicam-loaded gelatin microcapsule with enhanced bioavailability, a gelatin microcapsule encapsulated ethanol and piroxicam has been formulated by using gelatin as a water-soluble polymer shell. The aqueous solubility and bioavailability of piroxicam in piroxicam-loaded microcapsule in rats were then evaluated compared to piroxicam powder. The piroxicam-loaded gelatin microcapsule spherical in shape with smooth surface showed the geometric mean diameter of about 19 μm. It had the piroxicam solubility of about 1.87 mg/ml and the amount of ethanol of about 4.37 μg/mg. Furthermore, it gave significantly higher total plasma concentrations, C_{max} and area under the blood concentration–time curve (AUC) of piroxicam in rats than did piroxicam powder, indicating that the drug from gelatin microcapsule could be more orally absorbed in rats. In particular, the AUC of piroxicam in gelatin microcapsule was significantly about 2 fold increased compared to piroxicam powder. This enhanced oral relative bioavailability of piroxicam in gelatin microcapsule was contributed by the marked increase in the absorption rate of piroxicam due to the improved solubility of piroxicam. Thus, the piroxicam-loaded gelatin microcapsule developed using spray-drying technique with gelatin, sodium lauryl sulfate and ethanol would be useful to deliver piroxicam in a pattern that allows fast absorption in the initial phase, leading to better absorption.

Key words piroxicam; gelatin microcapsule; solubility; bioavailability

Piroxicam [4-hydroxyl-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide], a non-steroidal anti-inflammatory agent, is widely used in treatment of moderate pain and fever. However, it is a drug with low water solubility and high membrane permeability included in class 2 of the Biopharmaceutical Drug Classification System. It is absorbed slowly and gradually through the gastrointestinal tract and consequently the onset of the analgesic and anti-inflammatory actions is delayed. Various formulations of poorly water-soluble piroxicam such as emulsifying liposphere, poly(lactic glycolic acid) (PLGA) microsphere, microencapsulation, inclusion complex, HPMC microsphere and solid dispersion were developed to improve the solubility and dissolution of piroxicam.

Recently, we developed a new oral dosage form termed ‘gelatin microcapsule’ for increasing the dissolution rate of poorly water-soluble ibuprofen. The poorly water-soluble ibuprofen encapsulated with water-soluble gelatin is easily soluble or dispersed in gastrointestinal tract after oral administration, which leads to improving the bioavailability of poorly water-soluble drug.

In this study, to develop a piroxicam-loaded gelatin microcapsule for improving the oral bioavailability of piroxicam, a gelatin microcapsule encapsulated ethanol and piroxicam has been formulated by using gelatin as a water-soluble polymer shell. The bioavailability of piroxicam-loaded microcapsule in rats was then evaluated compared to piroxicam powder. Sodium lauryl sulfate is an anionic surfactant commonly used in pharmaceutical preparations. Previously, it has been employed to prevent microcapsules from attaching to the inner wall of spray drying chamber and to produce free-flowing powder.

MATERIALS AND METHODS

Materials Piroxicam and gelatin were supplied from Whawan Pharm. Co., Ltd. (Seoul, South Korea) and Sammi Co., Ltd. (Anyang, South Korea), respectively. Ethanol and sodium laurylsulfate were obtained from Ducksan Chemical (Seoul, Korea) and Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), respectively. All other chemicals were of reagent grade and used without further purification.

Preparation of Piroxicam-Loaded Gelatin Microcapsules A Büchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of gelatin microcapsule. First, 4 g gelatin was dissolved in 30 ml water to obtain aqueous gelatin solution. Piroxicam (1 g) was dissolved in 70 ml alkalic ethanol (0.1 M NaOH) to obtain the piroxicam solution. Sodium lauryl sulfate (0.6 g) and this piroxicam solution were then added to aqueous gelatin solution one after another. The resulting clear solution was prewarmed to 50 °C. The resulting solution was delivered to the nozzle at a flow rate of 5 ml/min using a pump and thereafter spray-dried at 105 °C inlet temperatures. The pressure of spray air was 5 kg/cm². The flow rate of drying air was maintained at the aspirator setting of 10 which indicated the pressure of aspirator filter vessel of ~30 mbar. The direction of air flow was the same as that of sprayed products. The diameter of nozzle was 0.7 mm.

The shape and surface of piroxicam-loaded gelatin microcapsule were examined using a scanning electron microscope (S-4100, Hitachi, Japan). The microcapsules were loaded on

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the specimen stub via double-side sticky tape and coated with gold (Hitachi Iron sputter, E-1030) for 30 min at 100—
200 mTorr in a shutter coater before taking photographs at an accelerating voltage of 2.4 kV.\(^{16}\)

**Aqueous Solubility of Piroxicam in Microcapsules**

For the determination of aqueous solubility of piroxicam, excessive amount of gelatin microcapsule (about 50 mg) was added to 3 ml of water, shaken in water bath for 3 d and filtered through membrane filter (0.45 μm). The concentration of piroxicam in the resulting solution was then analyzed by HPLC (Jasco UV-975, Japan) equipped with an Inertsil ODS-3 C18 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 with glacial acetic acid. The eluent was monitored at 330 nm with a flow rate of 1.5 ml/min.\(^{14}\)

**Determination of Ethanol Content in Microcapsule**

The various volumes (0.5, 1, 2, 4, 8 ml) of ethanol stock solution (0.1 g/ml) and acetonitrile (150 μl) as an internal standard were mixed and adjusted to 100 ml with deionized water in a volumetric flask for the preparations of standard solutions. About 250 mg of each alcoholic microcapsule was accurately weighed and dissolved in 10 ml acetonitrile–deionized water mixture (1.5 μl/ml) in an Eppendorf tube. The ethanol content in microcapsules was determined in a gas chromatography with a porapak Q, Chromosorb 101 column. Nitrogen gas was used as a carrier gas. The temperature of chromatography with a porapak Q, Chromosorb 101 column.

**Pharmacokinetics**

In Vivo Experiments: Male Sprague-Dawley rats weighing 300±20 g were fasted for 24—36 h 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 gelatin microcapsule (57 mg/kg equivalent to 10 mg/kg piroxicam), respectively. All animals care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989 and revised in 1999 by the Society of Toxicology.\(^{18}\)

Administration and Blood-Collecting: Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube 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 piroxicam-loaded gelatin microcapsule were filled in small hard capsule (#9, Suheung Capsule Co., Ltd., Seoul, South Korea), and orally administered to rats in each group, respectively. Half milliliter 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, U.S.A.).\(^{19,20}\)

Blood Sample Analysis: Plasma (0.18 ml) was mixed with 0.02 ml of ethanolic solution containing naproxen (0.2 μg/ml), as an internal standard and 0.02 ml of 60% perchloric acid. It was then centrifuged at 12000 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.\(^{5,23}\)

**RESULTS AND DISCUSSION**

On drying the gelatin dissolved in an ethanol–water cosolvent system on a rotary evaporator, ethanol and water evaporate simultaneously and gelatin is finally dried. However, microcapsules containing ethanol in the gelatin shells are produced by spray-drying the above solution as follows. Spray-drying the gelatin dissolved in ethanol–water mixture through a fluid pressure nozzle into the drying chamber at an appropriate temperature, ethanol and water are initially evaporated within the chamber of the spray dryer at the same time. However, as the atomized liquid droplets contact the hot drying air for a little longer, the concentration of gelatin begins to increase near the surface of liquid droplets and the water content on the surface of droplets decreases very rapidly as water and ethanol evaporate. As a result, a concentrated gelatin layer is formed on the surface of droplets. Water is continuously dried through the concentrated gelatin layer, but ethanol scarcely passes through this layer due to the extremely low diffusion coefficient of ethanol in concentrated gelatin layer.\(^{22—24}\)

Therefore, the concentrated gelatin will act as a semipermeable membrane, permitting continual water loss by diffusion but effectively retaining ethanol. Finally, the gelatin is solidified and ethanol is captured inside the gelatin shell and gelatin microcapsule is produced. Employing the same principle of producing the powder alcohol, piroxicam-loaded gelatin microcapsule could be prepared by spray-drying the solution of piroxicam and gelatin simultaneously dissolved in ethanol–water cosolvent system (Fig. 1). Piroxicam-loaded gelatin microcapsule is a solid form of microcapsules simultaneously containing ethanol and piroxicam in water-soluble gelatin shell.

The scanning electron micrographs of piroxicam-loaded gelatin microcapsule were illustrated in Fig. 2. The gelatin microcapsule (Fig. 2A) was spherical in shape with smooth surface.\(^{16}\) Figure 2B demonstrated that a cross-sectional view of gelatin microcapsule showed the large inner cavity containing ethanolic drug solution in a gelatin shell. The geometric mean diameter of piroxicam-loaded gelatin microcapsule was 19.13 ± 7.5 μm. This piroxicam-loaded gelatin microcapsule had the piroxicam solubility of about 1.87 mg/ml and the amount of ethanol of about 4.37 μg/ml. It was reported that the solubility of piroxicam powder was about 82.3 μg/ml.\(^{23}\)

Thus, this gelatin microcapsule improved about 2000-fold solubility of piroxicam. Furthermore, alkaline ethanol encapsulated in gelatin microcapsule could
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were not significantly different from those from piroxicam powder. Our results suggested that piroxicam-loaded gelatin microcapsule would be useful to deliver piroxicam in a pattern that allows fast absorption in the initial phase, leading to better absorption.

CONCLUSION

Taken together, it was concluded that the piroxicam-loaded gelatin microcapsule with the piroxicam solubility of about 1.87 mg/ml and the amount of ethanol of about 4.37 mg/ml gave significantly higher initial plasma concentrations, $C_{\text{max}}$ and AUC of piroxicam than did piroxicam powder, indicating that the drug from gelatin microcapsule could be more orally absorbed in rats. Thus, the piroxicam-loaded gelatin microcapsule developed using spray-drying technique with gelatin, sodium lauryl sulfate and ethanol was a more effective oral dosage form for poorly water-soluble piroxicam.

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