약제학회지(2005), 제 35 권 제 1 호
J. Kor. Pharm. Sci., Vol. 35, No. 1, 1-5 (2005)

The Effect of Solvents on Solid Dispersion of Ipriflavone with Polyvinylpyrrolidone *In Vivo*

Je Kyo Jeong, Yong San Ahn*, Byung Kwan Moon, Myung Kyu Choi*, Gilson Khang*,**,***†, John M. Rhee*,** and Hai Bang Lee*,*****

Research Center, Samchundang Pharm. Co. Ltd., Seoul 150-037, Korea
**Department of Polymer·Nano Science and Technology, Chonbuk National University, 664-14, Dukjin, Jeonju 561-756, Korea*
***Departmemt of Advanced Organic Materials Engineering, Chonbuk National University, 664-14, Dukjin, Jeonju 561-756, Korea*
****Nanobiomaterials Laboratory, Korea Research Institute of Chemical Technology, P. O. Box 107, Yuseong, Daejeon 305-600, Korea*
(Received November 9, 2004 · Revised December 29, 2004 · Accepted January 15, 2005)

ABSTRACT–Ipriflavone is a synthetic flavonoid derivate that improves osteoblast cell activity inhibiting bone resorption. In order to improve the bioavailability, solid dispersions of ipriflavone with PVP (poly-N-vinylpyrrolidone, MW=40,000 g/mole) were prepared by a spray-drying method. During the manufacturing of solid dispersion, various solvents [ethanol (EtOH), acetonitrile, methylene chloride and cosolvent-EtOH:acetone=1:1] were used to dissolve the ipriflavone and PVP. Scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) were used to evaluate the physicochemical interaction between ipriflavone and PVP. Particle size, crystallinity and the area of the endotherm (ΔH) of solid dispersed ipriflavone using the acetonitrile as solvent were much smaller than those of the other preparation types. Bioavailability of ipriflavone *in vivo* was changed by solvents. When considering the result of *in vivo* test, solid dispersion of ipriflavone using the acetonitrile as solvent showed the best choice.

Key words–Ipriflavone, Solid dispersion, Bioavailability, Various Solvents

Because solubility of drug is directly connected with bioavailability, it is important that particle size of drug must be decreased in order to increase the solubility of drug.[1,2] The method for improving solubility involves prodrug, inclusion complex, microencapsulation, microemulsion, solid dispersion, and so on.[3-15] It was possible that poorly water soluble drugs could be changed to high soluble and bioavailable drugs using those methods. One of these methods was a reduction of the particle size of drugs.[16,17] Although the reduction of particle size could be easily and directly achieved by conventional grinding and ball milling, aggregating powder and nano scale air bubbles in the powder could be disturbing the dissolution behavior of drugs.[18,19]

To overcome this problem, solid dispersion which had high dissolution rate and very large surface area in polymeric vehicles has been suggested.[20,21] When sulfathiazole was mixed with urea, the release rate of sulfathiazole in this mixture had dramatically increased comparing with only sulfathiazole.[22] Solid dispersion process was uniformly mixing method that the solvent was eliminated by freeze-dry or spray-dry, after poorly water-soluble drug and water-soluble polymer are dissolved in solvent. During the process of solid dispersion, crystallinity and particle size of drug decreased. As a result of this processing, dissolution rate and bioavailability could be increased in the body.[23,24]

It has been well known that ipriflavone (IP, 3-phenyl-7-isopropoxy-4H-1-benzopyran-4-one), which has been used in the treatment of osteoporososis, is a poorly water-soluble drug with an extremely low absorption rate in the body (below 1 μg/ml). Poly-N-vinylpyrrolidone (PVP) used to make solid dispersion is amorphous polymer, so it is widely used to decrease crystallinity of drug.[25-27]

In our previous study, we investigated the comparative bioavailability of IP by pharmaceutical preparation types,[1] effect of molecular weights and mixture ratios of PVP on the bioavailability of IP solid dispersion,[8] and preparation and characterization of solid dispersion of IP with PVP.[17] The aims of this study are (1) to prepare the solid dispersions using various solvents such as ethanol (EtOH), acetonitrile, methylene chloride and cosolvent of EtOH:acetone and (2) to observe *in vivo* absorption of IP in solid dispersion samples. Also, to evaluate the physicochemical property between IP and PVP, samples were characterized with scanning electron microscopy (SEM) and differential scanning calorimetery (DSC).

†본 논문에 관한 문의는 이 저자에게로
Tel : 063)270-2336, E-mail : gskhang@chonbuk.ac.kr

Experimental

Materials

IP was purchased from Dongbang Chem. Co., Korea and PVP (K-30) was obtained from Hongsung Pharm. Co., Korea with a weight-average molecular weight of 40,000 g/mol. Various solvents such as ethanol, acetone, methylene chloride and acetonitrile were used as a HPLC grade.

Preparation of solid dispersions

To make the solid dispersion, same weight (20 mg) of two components (IP and PVP) was dissolved in solvent at the room temperature and then spray-dried to disperse IP in PVP by fluidized bed coater (Uniglatt, Glatt Co., Germany) under the condition of 12~20 ml/min pump speed, 40~70°C inlet air temperature, 40~45°C outlet air temperature, and 20~40 psi spraying air pressure. In this study, the samples were named SIP-MeCN (acetonitrile), SIP-MC (methylene chloride), SIP-EtOH (ethanol) and SIP-Co (ethanol:acetone=1:1) as used solvents, respectively. In these preparations, the weight ratio of the drug to the water soluble polymer was 5:5 (w/w). After all the samples were prepared, they were stored for 12 hrs at −20°C and subsequently freeze-dried for 24 hrs in order to remove any residual solvents.

SEM

SEM (model S-2250N, Hitachi, Japan) was used to examine the morphology and particle size of IP solid dispersions. All samples for SEM were attached on metal stub double-sided tape and coated with platinum for 90 seconds under argon atmosphere using plasma sputter (SC 500K, Emscope, UK). The obtained photographs were examined at a magnification ratio of ×800.

DSC

Thermal properties of IP and solid dispersion samples were determined by DSC (2910, TA instrument, USA). The endothermic heats associated with the melting of the IP crystals were analyzed. After calibrating DSC with an indium standard, samples (5~15 mg) were weighed in aluminum pans and DSC analyses were carried out at a nitrogen flow of 50 ml/min and a heating rate of 2°C/min from 30 to 200°C. The endothermic energy was determined by measuring the peak areas.

In vivo test

The *in vivo* test was carried out on Sprague-Dawley rats (ca. 250~300 g, body weight, Toxicology Center Breeding Facility, Korea Research Institute of Toxicology, Daejeon, Korea) which were housed under specific pathogen free conditions. A single dose of 50 mg/kg was given orally to each rat (n=3). Blood samples (ca. 200 μl) were collected from the tail vein at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hrs after administration and plasma was prepared by centrifugation at 12,000 rpm for 5 min. The plasma samples were stored at −20°C until further processing.

The drug concentrations in the plasma were determined by high performance liquid chromatography (HPLC). The HPLC system was consisted of a UV detector (UV-2000, Thermo Separation Products, USA), a pump (P-2000, Thermo Separation Products), and an autosampler (AS-3000, Thermo Separation Products). The analytical columns consisted of a C_{18} Nova-Pak cartilage (Waters, USA) and an Inertsil ODS (5 μm, 250 × 4.6 mm ID, GL Sci. Inc., Japan). The mobile phase was a mixture of acetonitrile and distilled water (70:30 v/v). The UV wavelength selected for detection was 250 nm and the flow rate was 1.0 ml/min. Plasma proteins were precipitated using a triple volume of methanol, and aliquots (20 μl) of the supernatant were injected into the HPLC column.[1,8,17] The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule. The maximum plasma concentration (C_{max}) and the time to maximum concentration (t_{max}) were directly obtained from the observed values. All data were examined for their statistical significance of difference with Student's t-test ($P < 0.05$).

Results and Discussion

Morphology of the samples

SEM microphotographs of SIP-MeCN, SIP-EtOH, SIP-MC and SIP-Co were shown in Figure 1. After spray dry processing, all of the samples obtained smaller particle size than intact IP. It could be observed that most of the IP particles of SIP-EtOH, SIP-MC and SIP-Co were spherical shape, whereas the sample used MeCN as solvent was sharp shape. According to the difference of shape, it would be expected that the sample using MeCN as solvent has the unlike results from extra samples. Also, it could be observed that most of the particles of SIPs were not aggregated and agglomerated.

DSC

Figure 2 showed DSC thermograms of SIP-MeCN, SIP-EtOH, SIP-MC, SIP-Co and intact IP. ΔH of SIP-MeCN, SIP-EtOH, SIP-MC, SIP-Co and intact IP were observed at 40.7, 56.4, 60.7, 63.2 and 118 J/g, respectively. It could be observed that H of SIP-MeCN was smaller than those of SIP-EtOH, SIP-MC, SIP-Co and intact IP, that is to say, in the order of

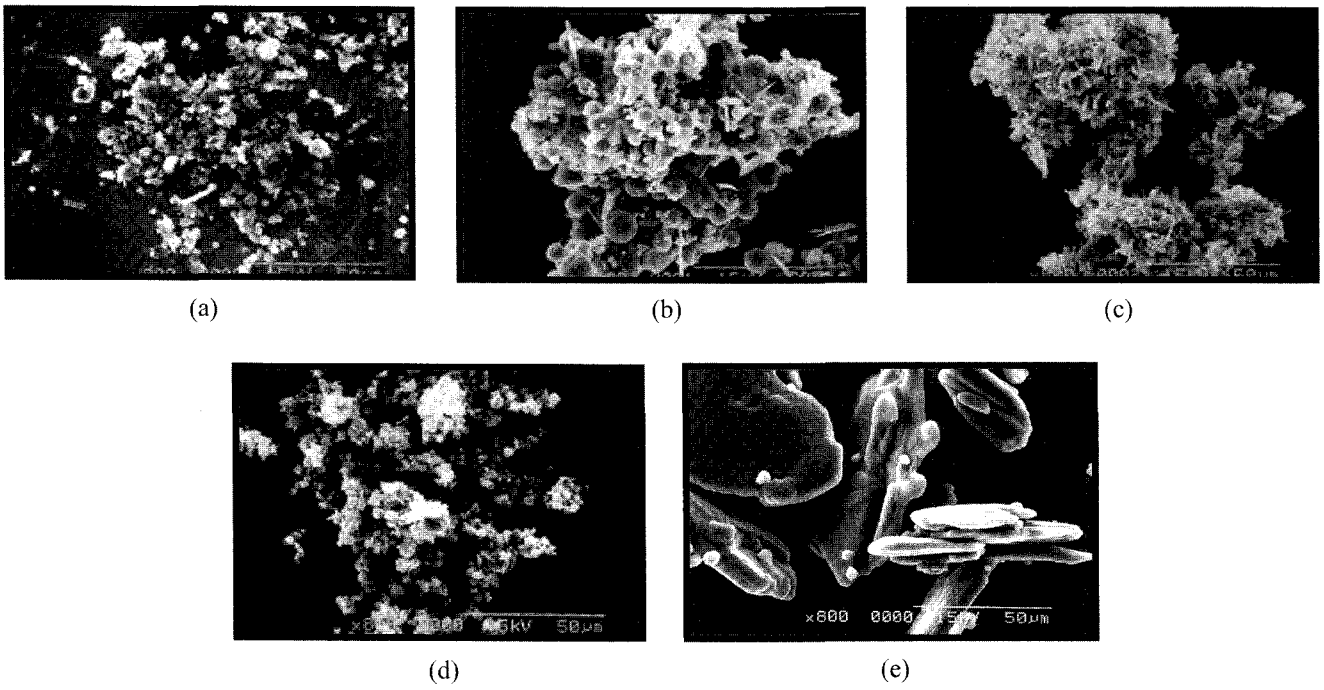

Figure 1–SEM photographs of (a) SIP-Co, (b) SIP-MC, (c) SIP-MeCN, (d) SIP-EtOH and (e) intact IP (original magnifications; ×800).

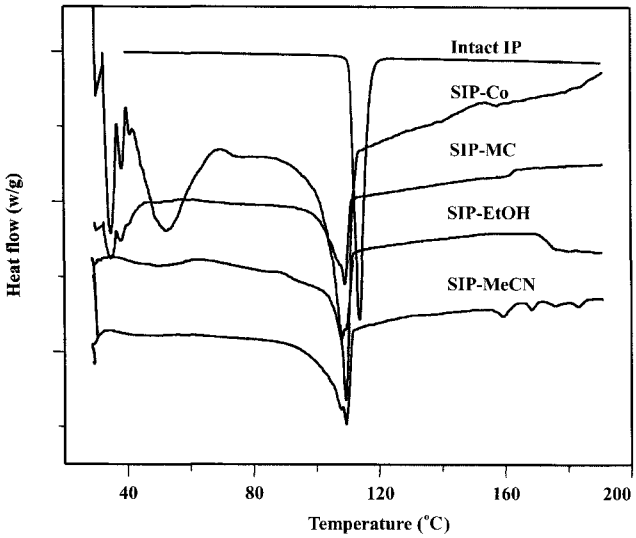

Figure 2–DSC thermograms of SIP-MeCN, SIP-Co, SIP-MC, SIP-EtOH and intact IP.

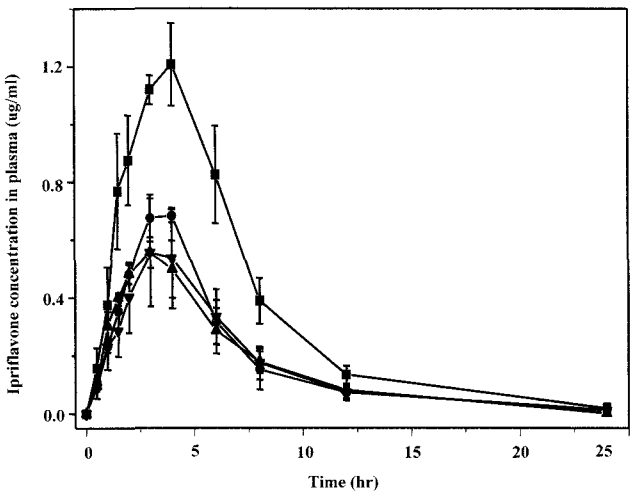

Figure 3–Plasma concentration-time curves of IP following oral administration of SIP-MeCN (■), SIP-Co (●), SIP-MC (▲) and SIP-EtOH (▼) in SD rats. Values are mean±S.E. (n=3).

intact IP > SIP-MC ≥ SIP-Co ≥ SIP-EtOH > SIP-MeCN.

Analysis of ipriflavone concentration in blood plasma

As mentioned in previous studies,[1,8] the retention time of IP was 11 min. In the blank plasma samples, there were no peaks that interfered at the retention time of IP. The limits of quantitation (LOQ) was 10 ng/ml when it was defined as the same concentration of IP resulting in an signal-to-noise ratio (S/N ratio) of 5. The standard curve was linear over the range 0.1~5 μg/ml. The correlation coefficients were greater than 0.999 for IP and the coefficients of variation for precision and accuracy were smaller than 10%. Also the absolute recovery was over 90%. Therefore, the present HPLC method is a rapid and simple assay procedure for IP in plasma.

***In vivo* test**

Figure 3 showed IP concentration profiles in blood plasma of SIP-MeCN, SIP-cosolvent, SIP-MC and SIP-EtOH. SIP-

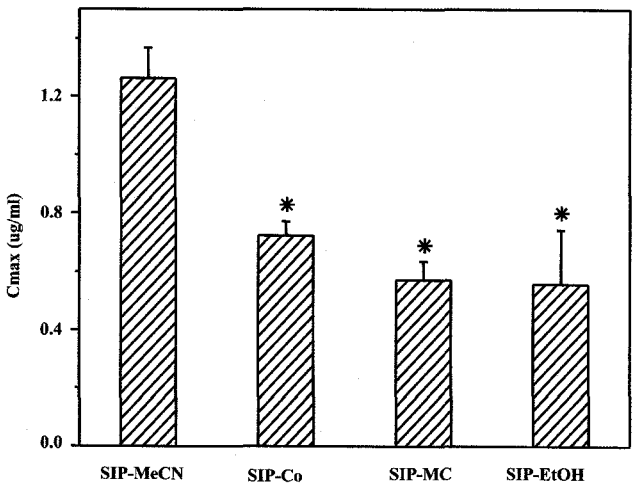

Figure 4–Maximum plasma concentration IP following oral administration of SIP-MeCN, SIP-Co, SIP-MC and SIP-EtOH in SD rats. Values are mean±S.E. (n=3).
*Significantly different from SIP group (P<0.05).

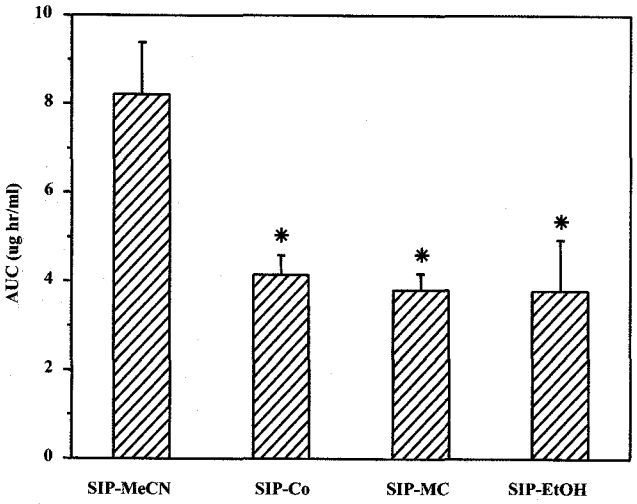

Figure 5–Areas under the plasma concentration curve of IP following oral administration of SIP-MeCN, SIP-Co, SIP-MC and SIP-EtOH in SD rats. Values are mean±S.E. (n=3).
*Significantly different from SIP group (P<0.05).

MeCN showed significantly higher absorption than those of SIP using cosolvent, ethanol, and methylene chloride. In the case of intact IP administration, IP concentrations in plasma were under LOQ.[1] Figures 4 and 5 showed the C_{max} and the AUC following oral administration of SIP-MeCN, SIP-cosolvent, SIP-MC and SIP-EtOH. In Figure 4, the C_{max} was significantly increased in SIP-MeCN (1.263 ± 0.105 μg/ml, $P < 0.05$), compared with those in SIP-cosolvent (0.723 ± 0.049 μg/ml), SIP-MC (0.572 ± 0.063 μg/ml), and SIP-EtOH (0.558 ± 0.186 μg/ml), that is to say, the C_{max} of SIP was about 1.8, 2.2 and 2.3 times higher than those of SIP-cosolvent, SIP-MC and SIP-EtOH, respectively. In Figure 5, the AUC was significantly increased in SIP-MeCN (8.128 ± 1.174 μg·hr/ml, $P < 0.05$), compared with those in SIP-cosolvent (4.143 ± 0.436 μg·hr/ml), SIP-MC (3.808 ± 0.374 μg·hr/ml) and SIP-EtOH (3.791 ± 1.147 μg·hr/ml), that is to say, the AUC of SIP was about 2, 2.2 and 2.2 times higher than those of SIP-cosolvent, SIP-MC and SIP-EtOH, respectively. This result indicated bioavailability could be upgraded by using acetonitrile as solvent.

Evaporation rate and interaction among IP, PVP and solvents had an effect on morphology of samples. Similar investigations have been observed for itraconazole-Eudragit E 100, nifedipine-hydroxypropylmethylcellulose, and piroxicam-PVP solid dispersions with significantly improved bioavailability.[16] In general, the process of the crystallization of a highly crystalline drug during the processing of the solid dispersion is largely divided into two processes; (a) the creation of the crystal nucleus and (b) the growth of the crystal.[21] According to the result of DSC, it was expected that the interaction of IP with PVP occurred decrease of crystallinity. PVP which is amorphous polymer make it possible that IP transfer the territory of amorphous. This result is identical with the result of DSC.

In conclusion, we had demonstrated that solid dispersion of IP with PVP was effective for the improvement of bioavailability. It seems that used solvent play important roles in *in vivo* absorption.

Acknowledgements

This work was supported by grants from the Korea Ministry of Science and Technology.

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