Bioavailability of Puerarin-phospholipid Solid Dispersion in Rats

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The purpose of this investigation is to evaluate the bioavailability in rats after oral administration of puerarin or puerarin-phospholipid solid dispersion. A simple and sensitive HPLC method was developed for determination of puerarin in rat plasma. It was shown that its plasma concentration reached a peak of 0.35 μg/ml at 0.64 h after oral administration (50 mg/kg). However, after intake of puerarin-phospholipid solid dispersion, a peak of 0.78 μg/ml occurred at a later time, 1.06 h. There was a significant difference in the mean area under the concentration-time curves (AUC) between the free drug and the puerarin-phospholipid solid dispersion.

Pueraria Lobata Ohwi is frequently used in traditional Chinese medicine as a remedy for cardiac disease and puerarin, the main constituent of this plant, has been used in treatment of coronary disease and high blood pressure[1]. However, according to studies on puerarin[2-3], the absorption of it *in vivo* after oral intake was poor because of its low hydrophilicity and lipophilicity which could be a major limitation for its clinical use.

Phospholipids can improve the hydrophilicity and lipophilicity of some drugs such as silimarin, griseofulvin, baicalin and ethopropazine, and enhance their oral absorption when phospholipids and drugs existed in the forms of complexes or coprecipitates or solid dispersions[4-7]. To improve puerarin's oral absorption, we prepared puerarin-phospholipid solid dispersion using the method of solvent evaporation. In the present paper, we investigated the bioavailability of puerarin-phospholipid solid dispersion following oral administrating to rats by a new HPLC method. Different from the published HPLC methods for puerarin assay in plasma, such as using fluorescence detection[8-10] or using ultraviolet detection which needed a troublesome sample treatment[11], the simple and sensitive reversed-phase HPLC method described in this paper was established by injecting an aliquot of supernatant liquid after deproteinization with 6% perchloric acid solution.

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The reference standard of puerarin used in the assay was obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China. Puerarin for animal studies and for preparing puerarin-phospholipid solid dispersion was purchased from XieHe Pharmaceutical Factory in Beijing and the soy phospholipid was purchased from Taiwei Pharmaceutical Co. in Shanghai. Methanol used was of HPLC grade and other reagents were analytically pure. Male Wistar rats weighing 210±30 g were obtained from the Test Animal Center of Shandong University and fasted overnight before being used in the animal studies. An Agilent 1100 HPLC system comprising of a chemstation, a vacuum degasser, a thermostatted column compartment, a diode array and multiple wavelength detectors and a quaternary pump was used in this investigation. The HPLC conditions were as follows: column-Phenomenex 4.6X250 mm Luna 5 μm ODS C18; mobile phase-metha-

nol:0.4% citric acid solution (21:79,v/v); detection wavelength-250 nm, flow rate-1.0 ml/min and column temperature-25°.

One gram of puerarin was dissolved in 100 ml of ethanol and treated with 3 g of soy phospholipid. The resulting solution was refluxed for about 2 h at 50° and then ethanol was collected by low pressure distillation, the precipitate was dried by vacuum desiccation and collected. The product was 3.94 g of puerarin-phospholipid solid dispersion in the form of a yellowish powder.

Puerarin or puerarin-phospholipid solid dispersion was dispersed in 0.5% aqueous solution of sodium carboxymethyl cellulose (CMC-Na). Each rat received a 50 mg/kg oral dose of puerarin (or a 200 mg/kg oral dose of puerarin-phospholipid solid dispersion). Blood samples were withdrawn from the subclavian vein sinus at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h following administration. Plasma was separated by centrifugation at 2500 rpm for 10 min and stored in a freezer until analysis.

Half a milliliter of 6% perchloric acid solution was added to 0.5 ml of plasma and the resulting mixture was vortexed for 5 min, then centrifugated at 15 000 rpm for 10 min. An aliquot (20 μl) of supernatant was injected into the HPLC column. The samples for the calibration curve were prepared similarly, except that a stock solution of puerarin in 50% methanol was added to the blank plasma at concentrations ranging from 0.1 to 5.0 μg/ml. By the least-squares analysis of peak area (A) against the corresponding concentration (C) of puerarin, a calibration curve was obtained. The regression equation was A=73.38C+1.292, r=0.9996.

Under the assaying conditions, the recoveries of puerarin extracted from plasma at the concentrations such as 0.1, 1.0, 5.0 μg/ml were more than 80% when compared with the corresponding puerarin solution. Plasma standards prepared over this concentration range exhibited an assay precision (RSD%) ranging from 3.4% to 8.7%. The limit of quantification of puerarin was 0.08 μg/ml and the within-day and between-day precision (RSD%) were less than 7.8% and 8.7%, respectively. A typical chromatogram of puerarin in plasma is shown in fig 1.

The data of the animal studies were treated with the Practical Pharmacokinetic Program 3p97[12], and the plasma concentration-time profile of puerarin was best described by a two-compartment model with lag-time and first-order absorption. After oral administration of puerarin and its phospholipid solid dispersion (n=8), plasma levels of puerarin

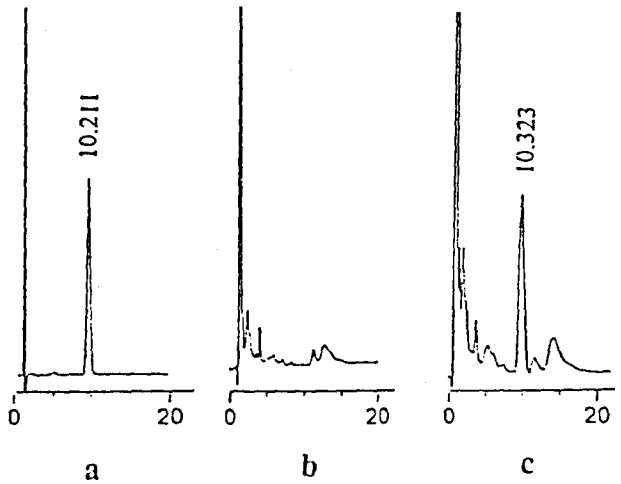

Fig. 1: The HPLC chromatograms of puerarin in plasma. The letters (a), (b) and (c) represent HPLC chromatograms of puerarin standard, drug-free plasma and plasma spiked with puerarin respectively. The retention time of puerarin is about 10 min.

were detectable only up to 8 h in rats given the free drug, but 12 h in rats given the solid dispersion. Puerarin plasma concentrations were much higher in rats given the solid dispersion than those in rats given the free drug at every sampling time, and the elimination of the solid dispersion was slower when compared with the free drug. All these resulted in a significant increase in AUC (2.747 vs 7.026 μg-h/ml) ($P<0.05$). The relevant pharmacokinetics parameters are listed in Table 1 (the results were expressed as mean ± standard deviation). It was concluded that the bioavailability of

TABLE 1: ORAL PHARMACOKINETIC PARAMETERS OF PUERARIN AND ITS PHOSPHOLIPID SOLID DISPERSION.

Parameters	Puerarin	Solid dispersion
AUC (μg-h/ml)	2.74±1.03	7.03±2.15*
CL (s)	18.0±4.12	7.12±2.24*
Cmax (μg/ml)	0.35±0.18	0.78±0.32
Tmax (h)	0.64±0.21	1.06±0.24

AUC, Cmax, Tmax, CL represent respectively the mean area under the concentration-time curves, the maximum concentration of drug in plasma, the peak time of drug in plasma and the total body clearance of drug. Statistically significant difference from puerarin at *$p<0.05$ were tested using a paired t-test.

puerarin in its phospholipid solid dispersion was enhanced.

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Preparation and Characterization of Agglomerates of Flurbiprofen by Spherical Crystallization Technique

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Flurbiprofen is an analgesic and antiinflammatory drug with poor water solubility and compressibility. Flurbiprofen conventional drug crystals were converted into spherical crystal agglomerates via the spherical crystallization technique using acetone-water-hexane solvent system. The various parameters optimized were type, amount and mode of addition of bridging liquid, temperature, and agitation speed to get maximum amount of spherical crystals. These were characterized for micromeritic properties (particle size and shape, flowability), packability (bulk density), wettability (contact angle) and compressibility. It was revealed from the study that spherical agglomerates exhibited improved flowability, wettability and compaction behaviour.

One of the most revolutionary technologies in the manufacture of solid dosage forms is tableting by direct compression. It is economical, facilitates processing without the need for moisture and heat and only few procedures are involved. In the direct compression method it is necessary to increase the flowability and compressibility of the bulk powder in order to have sufficient mechanical strength of the compacted tablets[1]. More recently, a modified crystalline technique has been adopted for the development of directly compressible drugs. This technique, also known as spherical crystallization, is a particle engineering technique by which crystallization and agglomeration can be carried out simultaneously in one step to transform crystals directly into a compacted spherical form[2]. This technique as the name indicates, provides crystalline agglomerates that are spherical in shape, which exhibit excellent micromeritic properties (flowability, packability, compressibility and wettability). This technique has been used to modify the properties of many drugs such as fenbufen[3], ibuprofen[4], furosemide[5], indomethacin[6], ami-

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