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Pharmacokinetics and correlation between *in vitro* release and *in vivo* absorption of bio-adhesive pellets of panax notoginseng saponins

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[ABSTRACT] The present study was designed to prepare and compare bio-adhesive pellets of panax notoginseng saponins (PNS) with hydroxy propyl methyl cellulose (HPMC), chitosan, and chitosan : carbomer, explore the influence of different bio-adhesive materials on pharmacokinetics behaviors of PNSbio-adhesive pellets, and evaluate the correlation between *in vivo* absorption and *in vitro* release (IVIVC). In order to predict the *in vivo* concentration-time profile by the i*n vitro* release data of bio-adhesive pellets, the release experiment was performed using the rotating basket method in pH 6.8 phosphate buffer. The PNS concentrations in rat plasma were analyzed by HPLC-MS-MS method and the relative bioavailability and other pharmacokinetic parameters were estimated using Kinetica4.4 pharmacokinetic software. Numerical deconvolution method was used to evaluate IVIVC. Our results indicated that, compared with ordinary pellets, PNS bio-adhesive pellets showed increased oral bioavailability by 1.45 to 3.20 times, increased C_{max}, and extended MRT. What's more, the release behavior of drug in HPMC pellets was shown to follow a Fickian diffusion mechanism, a synergetic function of diffusion and skeleton corrosion. The *in vitro* release and the *in vivo* biological activity had a good correlation, demonstrating that the PNS bio-adhesive pellets had a better sustained release. Numerical deconvolution technique showed the advantage in evaluation of IVIVC for self-designed bio-adhesive pellets with HPMC. In conclusion, the *in vitro* release data of bio-adhesive pellets with HPMC can predict its concentration-time profile *in vivo*.

[KEY WORDS] Panax notoginseng saponins; Bio-adhesive pellets; Pharmacokinetics; *In vivo* and *in vitro* correlation

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Introduction

Panax notoginseng saponins (PNS) are the effective active substances of root of notoginseng [*Panax notoginseng* (Burk.) F.H.C hen], a kind of perennial herbaceous plants affiliated to Araliacede, with the main active ingredients being notoginseng saponin and ginseng saponin. Studies have reported that PNS have various activities such as effects against cerebrovascular ischemia, anti-arrhythmic effects, diastolic relaxing blood vessels, improving blood rheology and microcirculation, inhibiting platelet aggregation and thrombosis, and reducing hematic fat and resistance to atherosclerosis [1-5]. PNS have good water solubility, and the solubility and dissolution rate are not the main factors affecting drug absorption [6-7]. However, poor stability under stomach condition, low membrane permeability, and high molecular weight are the main factors resulting in poor bioavailability.

Bio-adhesive preparation as a new drug delivery system has become more and more popularly in recent years; it could extend the time of pharmaceutical preparations' effects on target sites, increase the contact with the absorption membrane, change membrane fluidity, and increase drug penetration to the intestinal epithelial cells, thus promoting the absorption of drugs, and improving drug oral bioavailability [8-9]. Therefore, studying bio-adhesive preparations is highly significant.

Several researchers have prepared the controlled-release formulations using enteric technology [10-11], micro-porous osmotic pump tablets [12] or pulsatile controlled-release tablets [13], in order to improve the oral bioavailability by avoiding PNS degradation in gastric acid. Our team has prepared

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bio-adhesive tablets to improve the oral bioavailability [14-16] by increasing the drug intestinal absorption time via adhesion to the gastrointestinal tract. What's more, the bio-adhesive formulations have sustained-release effect to a certain extent to avoid PNS degradation in gastric fluid to a certain extent. The present study was designed to prepare skeleton-type bio-adhesive pellets using bio-adhesive materials that could promote intestinal absorption of PNS. PNS bio-adhesive pellets had larger superficial area than bio-adhesive tablets, increasing the adhesion area, prolonging the contact time of drugs with mucous membrane, promoting the absorption of drug, and effectively improving drug bioavailability [17].

In the present study, the *in vitro* release behaviors of the bio-adhesive pellets were evaluated, and the drug concentrations in blood were analyzed following administration of different PNS bio-adhesive pellets with different bio-adhesive materials. The pharmacokinetic parameters were estimated, so that the effects of different bio-adhesive materials on *in vivo* absorption of PNS could be determined. Additionally, the correlation between *in vitro* release and *in vivo* absorption was analyzed in order to provide reference for future researches on dosage forms and clinical applications.

Materials and Methods

Animals

Male Sprague–Dawley (SD) rats weighing 250–300 g were obtained from Vital River Laboratories, Beijing, China. The animals were housed at temperature of (25 ± 1) °C and relative humidity of 45%–55% under 12 : 12 light : dark cycle. Before study, the rats were allowed to acclimate to the environment for 7 days. All studies in mice were performed in accordance with guidelines approved by the Ethics Committee of the Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC).

Drugs and chemicals

PNS extract (PNS extract contained 2.30% of notoginsenoside R1, 15.02% of ginsenoside Rg1, and 26.80% of Rb1, respectively, the batch number is 201304) was purchased from Wenshan Kangzhou bio-technique Co. Ltd. (Wenshan, Yunnan, China). Standards of Notoginsenoside R1 (NGR1), Ginsenoside Rg1 (GRg1), and Ginsenoside Rb1 (GRb1) were purchased from the National Institute of the Control of Pharmaceutical and Biological Products (Beijing, China). In addition, Digoxin (internal standard) was purchased from the National Institute of the Control of Pharmaceutical and Biological Products (Beijing, China). Carbomer was purchased from BF Goodrich (Cleveland, USA). Chitosan was purchased from Jinqiao Biochemistry Company (Taizhou, Zhejiang, China). HPMC (K4M) was purchased from Colorcon Company (Shanghai, China). Microcrystalline cellulose (MCC) (PH101) was purchased from BF Goodrich. Chitosan was purchased from Asahi Kasei Corporation (Tokyo, Japan). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (New Jersey, USA). Heparin sodium was purchased from Beijing Yaobei Biological and Chemical Reagents Company (Beijing, China).

Preparation of normal and bio-adhesive pellets of PNS

We prepared normal and bio-adhesive PNS pellets, according to the methods of our previous research [18], using three types of bio-adhesive materials: chitosan, HPMC, and chitosan: carbomer. We homogeneously mixed PNS with MCC and other bio-adhesive materials after sifting and used 30% NaCl to get soft materials with modest viscoelasticity and plasticity. The soft materials were passed through screw extruder to get the same diameter, irregular length, smooth, and dense strings, at the rotate speed of 35 r·min^{-1}; the strings were passed through spheronizator, and the extrudates were rolled into balls under the effect of friction force and centrifugal force. The pellets were dried at 60 °C. Normal pellets were prepared by the similar ratio and method, but without bio-adhesive materials.

Release testing of PNS pellets

Quantitative analysis by high-performance liquid chromatography (HPLC)

A Shimadzu (Japan) Class VP HPLC system with a Kromasil C_{18} (10 mm × 4.6 mm; 2.4 μm) (Akzo Nobel, Goteborg, Sweden), and UV detector was used for drug content analysis. Mobile phase was composed of aqueous (A) and water (B), and the gradient elution program was as follows: 0−10 min: 20%−40% A; and 10−25 min: 40%−20% A. The flow rate was set at 1 mL·min^{-1}, the measurement wavelength was set at 203 nm, the column temperature was set at 30 °C, and injection volume was 20 μL. The regression equation and correlation coefficients of standard curves were as follows: NGR1: A = 2 649.2C − 241.7 (r = 0.999 7); GRg1: A = 13 686.3C − 2 043.2 (r = 0.999 9); GRb1: A = 2 087.9C −538.4 (r = 0.999 9). A represents peak area, C represents drug concentration. The linearity ranges was 0.2−50 μg·mL^{-1}.

In vitro release testing

In vitro release testing was carried out in release medium (pH 7.4 phosphate- buffered saline (PBS) solution) at (37 ± 0.5) °C. 0.5 g of pellets were suspended in the rotative baskets with 500 mL of release medium. At pre-determined time intervals, aliquots of 2-mL solutions were withdrawn and filtered through 0.45-μm filters. The sample volumes were replaced with equal volume of the fresh medium. NGR1, GRg1, and GRb1 released from pellets were quantified by HPLC, three tests were performed for each sample and the mean values were used as the final results.

Analysis of elease kinetics

The release data of optimized formulation were fitted to different mathematical models to reveal the release mechanism from the pellets: Zero order (% cumulative drug release *vs* time), first order (log% drug release *vs* time), Higuchi model (% cumulative drug release *vs* square root of time), and Peppas exponential equation (log% drug release *vs* log time). All the curve fitting, simulation, and plotting were performed using commercially available Microsoft excel solver, and regression coefficient (r^2) values were calculated.