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 PNS bio-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 in 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 $C_{\text{max}}$, 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

[CLC Number] R969.1

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 Araliace, 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.
in order to improve the oral bioavailability by avoiding PNS degradation in gastric acid. Our team has prepared bio-adhesive tablets to improve the oral bioavailability 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 Jinjiao 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 spheronizer, 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 C₁₈ (10 mm × 4.6 mm; 2.4 µm) (Akzo Nobel, Göteborg, 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 release 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), Korsmeyer–Peppas model (% cumulative drug release vs log cumulative drug release), Higuchi model (% cumulative drug release vs log time), Higuchi–Korsemeyer–Peppas model (% cumulative drug release vs time), and Higuchi–Korsemeyer–Peppas model (% cumulative drug release vs square root of time).
time), and Peppas exponential equation \((\log \% \text{drug release vs log time})\). All the curve fitting, simulation, and plotting were performed using commercially available Microsoft excel solver, and regression coefficient \((r^2)\) values were calculated.

**In vivo pharmacokinetic study**

**Instrumentation and analytical conditions**

Method validation proved that the current assay can be successfully applied to the determination of NGR1, GRg1, and GRb1 after a single oral administration of 90 mg·kg\(^{-1}\) of PNS in three different kinds bio-adhesive pellets of PNS. The analytical column C\(_{18}\): Thermo C\(_{18}\) (10 mm × 4.6 mm; 2.4 μm) was used at 25 °C, with water containing 10 mmol·L\(^{-1}\) of ammonium acetate, 10% methol and 5% N-butylamine (A) and methanol (B) gradient as mobile phase at a flow rate of 0.5 mL·min\(^{-1}\). The elution was carried out as follows: 50%–95% B at 0–2.0 min; 95% B at 2.0–4.0 min; 95%–50% B at 4.0–6.0 min; and 50% B at 6.0–8.0 min. The injection volume was 20 μL and the partial loop with needle overfill mode was used for sample injection.

The mass spectrometer was operated in positive ionization mode using MRM to assess the three ginsenosides and Digoxin: \(m/z\) 955.7 → 775.4 for NGR1, \(m/z\) 823.6 → 643.6 for GRg1, \(m/z\) 1 132.1 → 365.7 for GRb1 and \(m/z\) 803.5 → 283.4 for Digoxin (IS), respectively. The optimized cone voltage and collision energy were 100 V and 22 eV for NGR1, 90 V and 24 eV for GRg1, 100 V and 30 eV for GRb1, and 50 V and 20 eV for IS, respectively. A spray voltage of 5 500 V was used, and the capillary temperature was set at 550 °C. The scanning range was selected as \(m/z\) 100→1 200: no interference was observed around target compound peaks. Data acquisition and processing were accomplished on a 3200 Q TRAP® mass spectrometer (Applied Biosystems, Waltham, Massachusetts, USA).

**Sample preparation**

20 μL of Digoxin (1 000 ng·mL\(^{-1}\) in methanol, IS) was added to 150 μL of plasma. The sample was mixed with 0.5 mL of acetone for 5 min and then centrifuged at 12 000 r·min\(^{-1}\) for 10 min. The supernatant was collected, transferred to a clean centrifuge tube, and evaporated to dryness at 60 °C. The resultant residue was dissolved in 150 μL of 80% methanol, vortexed for 3 min, and centrifuged at 12 000 r·min\(^{-1}\) for 5 min. The supernatant was collected, filtered through 0.22-μm microfiltration membrane, and injected onto HPLC-MS/MS system for analysis.

**Method validation**

**Specificity**

The specificity of the method was investigated by analyzing blood samples from healthy rats, spiked blood samples, and blood samples after intranasal administration of PNS pellets, to exclude any endogenous co-eluting interference.

**Linearity and LLOQ**

The ginsenoside concentrations were plotted against the ratio of ginsenoside peak area over that of Digoxin. A weighted \((1/\text{nominal concentration})\) least-square linear regression \((y = bx + a)\) was used to fit the curves. The least concentration of the calibration curve was considered LLOQ.

**Precision and accuracy**

The intra- and inter-day precision and accuracy of the method were determined by analyzing five replicates at low, medium, and high concentrations with the same analytical run on three consecutive days.

**Stability**

The stability of NGR1, GRg1 and GRb1 in blood samples was assessed at three quality control (QC) levels with five replicates under different storage and processing conditions, including 4 h storage at ambient temperature, freeze-thaw for three cycles, to determine long-term and post-preparative stability. The calibration curves were freshly generated during the stability assays.

**Administration of PNS and sample collection**

According to our double cycle and crossover experimental design, normal and bio-adhesive pellets were administered orally at a dose of 200 mg·kg\(^{-1}\) to six rats each group. All the rats were deprived of food but given free access to water for 12 h before the experiments. The blood samples (0.5 mL each time) were collected via orbit at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after administration. The plasma was separated after centrifugation for 10 min at 7 000 r·min\(^{-1}\) and stored at −20 °C until analysis.

**Correlation study between in vitro release and in vivo pharmacokinetic characteristics**

Deconvolution method was used to investigate the in vitro release and in vivo absorption correlation. The correlation coefficient was calculated with WinNonlin5.2 pharmacokinetic software.\(^{[19]}\) The cumulative release degree \(F\) represented in vitro release characteristics, the \(AUC\) at all time points represented the in vivo absorption, and the ratio of \(AUC\) at each point with \(AUC_{\text{in}}\) represented the Fraction Input (R).\(^{[20]}\)

**Results**

**In vitro drug release**

The cumulative release of PNS from the bio-adhesive pellets prepared with different bio-adhesive materials are shown in Fig. 1. The drug release rates were more than 80% for ordinary pellets and bio-adhesive pellets with chitosan within 20 min. The bio-adhesive pellets with HPMC had a better sustained release effect.

**Release kinetics**

The bio-adhesive pellets with HPMC and chitosan-carbomer were simulated with various mathematical models, such as zero order, first order, Higuchi, and Peppas kinetic models. The best fitted regression results were obtained with the Peppas model (Table 1). The release mechanism was then studied with the Peppas equation, indicatig a coupling of diffusion and erosion mechanism, suggesting that the drug release was controlled by more than one process.
In the present study, chromatogram examination ruled out any endogenous peaks at the retention times of NGR1, GRg1, GRb1, and Digoxin under optimized analytical conditions (Fig. 2).

**Method validation**

**Specificity**

In the present study, chromatogram examination ruled out any endogenous peaks at the retention times of NGR1, GRg1, GRb1, and Digoxin under optimized analytical conditions (Fig. 2).

**Linearity and LLOQ**

The regression equation (correlation coefficients) and linearity ranges of the analytes were as follows: $y = 0.090\, 3x + 3.804\, 6$ ($r = 0.997\, 7$) and $7.18−1\, 435.00\, \text{ng}\cdot\text{mL}^{-1}$ for NGR1; $y = 0.081\, 9x + 23.544\, 5$ ($r = 0.997\, 7$) and $6.75−1\, 350\, \text{ng}\cdot\text{mL}^{-1}$ for GRg1; $y = 0.002\, 4x + 1.615\, 2$ ($r = 0.995\, 3$) and $15.45−1\, 545.00\, \text{ng}\cdot\text{mL}^{-1}$ for GRb1. The LLOQ values were $7.18\, \text{ng}\cdot\text{mL}^{-1}$ for NGR1, $6.75\, \text{ng}\cdot\text{mL}^{-1}$ for GRg1, and $15.45\, \text{ng}\cdot\text{mL}^{-1}$ for GRb1, respectively. The results indicated a good linearity within the selected range. In addition, LLOQ with a ratio of signal to noise of more than 10 ($S/N > 10$) for the three analytes indicated acceptable assay accuracy and precision in blood sample analysis.

**Stability**

Stability values ($< 15\%$ of change from the baseline) indicated that all analytes were stable when stored at ambient temperature for $4\, ^\circ\text{C}$ and subjected to three freeze-thaw cycles ($−80\, ^\circ\text{C}$) (data not shown).

**Pharmacokinetic applications and plasma drug concentration-time curves of rats**

**Specificity**

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**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>NGR1</th>
<th>GRg1</th>
<th>GRb1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC</td>
<td>$y = 0.476, 6x + 2.038, 2$</td>
<td>$y = 0.503, 9x + 1.830, 7$</td>
<td>$y = 0.591, 9x + 1.368$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.984$</td>
<td>$R^2 = 0.9844$</td>
<td>$R^2 = 0.9566$</td>
</tr>
<tr>
<td>Chitosan : Carbomer</td>
<td>$Y = 0.593, 6x + 0.572$</td>
<td>$Y = 0.572, 5x + 0.678, 2$</td>
<td>$Y = 0.539, 6x + 0.721, 6$</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.996, 3$</td>
<td>$R^2 = 0.995, 1$</td>
<td>$R^2 = 0.984, 4$</td>
</tr>
</tbody>
</table>

**Fig. 1**  

In vitro release profiles of bio-adhesive pellets with different bio-adhesive materials. A: NGR1, B: GRg1, and C: GRb1.

**Table 1** Release kinetics simulated with the Peppas model

<table>
<thead>
<tr>
<th>Groups</th>
<th>NGR1</th>
<th>GRg1</th>
<th>GRb1</th>
</tr>
</thead>
<tbody>
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</tr>
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</tr>
<tr>
<td></td>
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<td>$R^2 = 0.995, 1$</td>
<td>$R^2 = 0.984, 4$</td>
</tr>
</tbody>
</table>
Pharmacokinetics

We investigated the pharmacokinetic properties of PNS in rats, the concentration-time curves of three components of six rats are shown in Fig. 3. The $AUC$ values of NGR1, GRg1 and GRb1 with bio-adhesive pellets were increased remarkably, compared with that of the normal pellets ($P < 0.05$), and the relative bioavailability data after oral administration of bio-adhesive pellets are shown in Table 2 for NGR1, Table 3 for GRg1, and Table 4 for GRb1. These results indicated that bio-adhesive formulation considerably improved the oral bioavailability of PNS. Compared with the normal pellets, the parameters of $T_{\text{max}}$ for NGR1, GRg1 and GRb1 were significantly increased ($P < 0.05$) and the parameters of $C_{\text{max}}$ for NGR1 and GRb1 were increased remarkably for the bio-adhesive pellets ($P < 0.05$), which revealed that there was a process of accelerating release or improving assimilation.

Correlations between in vitro release and in vivo pharmacokinetic characteristics

The correlations between in vitro release and in vivo pharmacokinetic characteristics were evaluated using the deconvolution method. The Fraction input was calculated with WinNonlin5.2 software, the results are shown in Tables 5–7.

The cumulative rate of in vitro release ($Q$) is as the dependent variable $Y$, Fraction Input ($R$) is as the independent Variable $X$, and the regression equations are as follows: $Y = 75.272X + 7.531$, $r = 0.9837$ (NGR1); $Y = 73.485X + 8.583$, $r = 0.9805$ (GRg1); and $Y = 65.929X + 10.402$, $r = 0.9603$ (GRb1). The results are shown in Fig. 4. When the degree of freedom ($df$) was $6 - 2 = 4$ and critical value ($r_4$, Fig. 3) $= 0.968$, the $P$ value of $r$ was $< 0.05$. The dose of oral administration of PNS was $90 \text{ mg·kg}^{-1} (n = 6)$.
Table 2  Pharmacokinetic parameters of Notoginsenoside R1 after oral administration of PNS normal and bio-adhesive pellets (200 mg·kg⁻¹) to rats (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HPMC</th>
<th>Chitosan</th>
<th>Chitosan : Carbomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax/(ng·mL⁻¹)</td>
<td>112.01</td>
<td>391.47**</td>
<td>272.1**</td>
<td>456.9**</td>
</tr>
<tr>
<td>Tmax/h</td>
<td>0.75</td>
<td>1.5**</td>
<td>1.0**</td>
<td>1.0**</td>
</tr>
<tr>
<td>MRT/min</td>
<td>24.38</td>
<td>26.9</td>
<td>13.34</td>
<td>6.43</td>
</tr>
<tr>
<td>AUC0ₜ/(ng·mL⁻¹·h)</td>
<td>31 982.5</td>
<td>83 419.0</td>
<td>58 282.9</td>
<td>70 968.4</td>
</tr>
<tr>
<td>AUC0∞/(ng·mL⁻¹·h)</td>
<td>51 038.6</td>
<td>139 949.1</td>
<td>70 601.7</td>
<td>75 564.8</td>
</tr>
<tr>
<td>Fr%</td>
<td>100.00</td>
<td>260.83**</td>
<td>182.23**</td>
<td>221.90**</td>
</tr>
</tbody>
</table>

Fr: relative bioavailability = (AUC0₂₄,test) × 100/(AUC0₂₄,control). Comparative pharmacokinetics profile of NGR1 in blood (**P < 0.01 between normal and bio-adhesive pellets).

Table 3  Pharmacokinetic parameters of Ginsenoside Rg1 after oral administration of PNS normal and bio-adhesive pellets (200 mg·kg⁻¹) to rats (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HPMC</th>
<th>Chitosan</th>
<th>Chitosan : Carbomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax/(ng·mL⁻¹)</td>
<td>513.8</td>
<td>1 099.5**</td>
<td>1 458.5**</td>
<td>1 556.1**</td>
</tr>
<tr>
<td>Tmax/h</td>
<td>0.75</td>
<td>1.5**</td>
<td>1.0**</td>
<td>1.0**</td>
</tr>
<tr>
<td>MRT/min</td>
<td>24.49</td>
<td>47.23</td>
<td>14.95</td>
<td>6.91</td>
</tr>
<tr>
<td>AUC0ₜ/(ng·mL⁻¹·h)</td>
<td>128 818.5</td>
<td>311 731.8</td>
<td>261 009.1</td>
<td>293 905.2</td>
</tr>
<tr>
<td>AUC0∞/(ng·mL⁻¹·h)</td>
<td>208 919.0</td>
<td>669 067.2</td>
<td>330 085.5</td>
<td>315 191.4</td>
</tr>
<tr>
<td>Fr%</td>
<td>100.00</td>
<td>320.25**</td>
<td>228.15**</td>
<td>202.62**</td>
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</table>

Fr: relative bioavailability = (AUC0₂₄,test) × 100/(AUC0₂₄,control). Comparative Pharmacokinetics profile of GRG1 in blood (**P < 0.01 between normal and bio-adhesive pellets).

Table 4  Pharmacokinetic parameters of Ginsenoside Rb1 after oral administration of PNS normal and bio-adhesive pellets (200 mg·kg⁻¹) to rats (n = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HPMC</th>
<th>Chitosan</th>
<th>Chitosan : Carbomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax/(ng·mL⁻¹)</td>
<td>4 977.8</td>
<td>11 883.9**</td>
<td>9549.5**</td>
<td>14 884.1**</td>
</tr>
<tr>
<td>Tmax/h</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5**</td>
<td>1.0</td>
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<tr>
<td>MRT/min</td>
<td>14.94</td>
<td>11.87</td>
<td>48.76</td>
<td>11.04</td>
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<tr>
<td>AUC0ₜ/(ng·mL⁻¹·h)</td>
<td>2 693 861</td>
<td>5 284 163.6</td>
<td>2 740 140.0</td>
<td>4 129 113.1</td>
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<tr>
<td>AUC0∞/(ng·mL⁻¹·h)</td>
<td>3 359 531</td>
<td>6 350 225.3</td>
<td>6 376 695.8</td>
<td>4 871 884.3</td>
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<tr>
<td>Fr%</td>
<td>100.00</td>
<td>189.02**</td>
<td>145.01*</td>
<td>189.8**</td>
</tr>
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</table>

Fr: relative bioavailability = (AUC0₂₄,test) × 100/(AUC0₂₄,control). Comparative Pharmacokinetics profile of GRb1 in blood (**P < 0.01 between normal and bio-adhesive pellets).

Table 5  Parameter of devolution of NGR1, GRG1, and GRB1 after oral administration of bio-adhesive pellet with HPMC to rats (n = 6)

<table>
<thead>
<tr>
<th>t/min</th>
<th>C/(ng·mL⁻¹)</th>
<th>AUC/(ng·min·mL⁻¹)</th>
<th>Fraction Input (R)</th>
<th>Q%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGR1</td>
<td>GRG1</td>
<td>GRB1</td>
<td>NGR1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>60</td>
<td>30</td>
<td>215.17</td>
<td>5 521.76</td>
<td>591.103</td>
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<tr>
<td>120</td>
<td>90</td>
<td>237.48</td>
<td>9 330.85</td>
<td>10 548.798</td>
</tr>
<tr>
<td>240</td>
<td>180</td>
<td>237.48</td>
<td>9 330.85</td>
<td>10 548.798</td>
</tr>
<tr>
<td>360</td>
<td>360</td>
<td>112.25</td>
<td>3 054.29</td>
<td>13 499.047</td>
</tr>
<tr>
<td>480</td>
<td>480</td>
<td>134.98</td>
<td>2 632.78</td>
<td>15 991.441</td>
</tr>
<tr>
<td>720</td>
<td>720</td>
<td>125.16</td>
<td>1 875.22</td>
<td>20 649.123</td>
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Fig. 4 The in vivo and in vitro correlation curves for NGR1, GRg1 and GRb1 in bio-adhesive pellets with HPMC. Q(%) represents the cumulative rates of in vitro release, R represents Fraction Input.

The in vivo and in vitro correlation curves for NGR1, GRg1 and GRb1 in bio-adhesive pellets with HPMC. Q(%) represents the cumulative rates of in vitro release, R represents Fraction Input.

When the degree of freedom (df) was 6−2 = 4 and critical value (r4, 0.05) was 0.811, the correlation coefficient of regression equation (r) was > r4, 0.05, which indicated that NGR1, GRg1 and GRb1 in bio-adhesive pellets with HPMC had a good correlation between in vitro release and in vivo absorption, demonstrating that we could predict the concentration-time data in vivo using the in vitro release data of bio-adhesive pellets with HPMC.

The cumulative rates of in vitro release (Q) is as the dependent variable Y, Fraction Input (R) is as the independent Variable X, and the regression equations are as follows:

\[ Y = 75.272x + 7.531, R^2 = 0.983 \]

\[ GRg1(HPMC) \]
\[ y = 73.485x + 8.583, R^2 = 0.980 \]

\[ GRb1(HPMC) \]
\[ y = 65.929x + 10.402, R^2 = 0.960 \]

The results are shown in Fig. 5. When the degree of freedom (df) was 6 − 2 = 4 and critical value (r4, 0.05) was 0.811, the correlation coefficient of regression equation (r) was > r4, 0.05, which indicated that NGR1, GRg1 and GRb1 in bio-adhesive pellets with HPMC had a good correlation between in vitro release and in vivo absorption, demonstrating that we could predict the concentration-time data in vivo using the in vitro release data of bio-adhesive pellets with HPMC.

The cumulative rates of in vitro release (Q) is as the dependent variable Y, Fraction Input (R) is as the independent Variable X, and the regression equations are as follows:

\[ Y = 86.252x + 25.094, r = 0.811 \]
\[ GRg1(HPMC) \]
\[ y = 79.724x + 23.587, r = 0.802 \]
\[ GRb1(HPMC) \]
\[ y = 70.11x + 24.387, r = 0.772 \]

Similarly, the cumulative rates of in vitro release (Q) is as the dependent variable Y, Fraction Input (R) is as the independent Variable X, and the regression equations are as follows:

\[ Y = 86.252x + 25.094, r = 0.811 \]

\[ GRg1(HPMC) \]
\[ y = 79.724x + 23.587, r = 0.802 \]
\[ GRb1(HPMC) \]
\[ y = 70.11x + 24.387, r = 0.772 \]

The results are shown in Fig. 6. When the degree of freedom (df) was 6 − 2 = 4 and critical value (r4, 0.05) was 0.811, the correlation coefficient of regression equation (r) was < r4, 0.05, indicating that NGR1, GRg1 and GRb1 in bio-adhesive pellets with chitosan: carbomer had a bad correlation between in vitro release and in vivo absorption, which demonstrated that we couldn’t predict the concentration-time data in vivo using the in vitro release data of bio-adhesive pellets with chitosan: carbomer.

Table 6 Parameters of devolution of NGR1, GRg1, and GRb1 after oral administration of bio-adhesive pellet with chitosan-carbomer (n = 6)

<table>
<thead>
<tr>
<th>C/(ng·mL⁻¹)</th>
<th>AUC/(ng·min·mL⁻¹)</th>
<th>Fraction Input (R)</th>
<th>Q%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGR1</td>
<td>GRg1</td>
<td>GRb1</td>
<td>NGR1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>44.125</td>
<td>2</td>
<td>169.189</td>
</tr>
<tr>
<td>60</td>
<td>456.988</td>
<td>2</td>
<td>155.140</td>
</tr>
<tr>
<td>90</td>
<td>185.868</td>
<td>7</td>
<td>786.238</td>
</tr>
<tr>
<td>120</td>
<td>310.294</td>
<td>3</td>
<td>1.252</td>
</tr>
<tr>
<td>240</td>
<td>56.597</td>
<td>2</td>
<td>252.284</td>
</tr>
</tbody>
</table>

Similarly, the cumulative rates of in vitro release (Q) is as the dependent variable Y, Fraction Input (R) is as the independent Variable X, and the regression equations are as follows:

\[ Y = 71.351x + 45.98, r = 0.398 \]

\[ GRg1(CTS - Carbomer) \]
\[ y = 68.176x + 42.198, r = 0.413 \]

\[ GRb1(CTS - Carbomer) \]
\[ y = 64.417x + 45.18, r = 0.772 \]

The results are shown in Fig. 6. When the degree of freedom (df) was 6 − 2 = 4 and critical value (r4, 0.05) was 0.811, the correlation coefficient of regression equation (r) was < r4, 0.05, indicating that NGR1, GRg1, and GRb1 in bio-adhesive pellets with chitosan: carbomer had a bad correlation between in vitro release and in vivo absorption, which demonstrated that we couldn’t predict the concentration-time data in vivo using the in vitro release data of bio-adhesive pellets with chitosan: carbomer.

**Discussion**

In a previous study, we have explored in vitro adhesion of pellets with different bio-adhesive materials using the tissue retention method, the results demonstrated that in vitro adhesion of HPMC, chitosan and chitosan: carbomer...
Table 7  Parameters of devolution of NGR1, GRg1 and GRb1 after oral administration of bio-adhesive pellet with chitosan (n = 6)

<table>
<thead>
<tr>
<th>t/min</th>
<th>C/(ng·mL⁻¹)</th>
<th>AUC/(ng·min·mL⁻¹)</th>
<th>Fraction Input (R)</th>
<th>Q(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGR1</td>
<td>GRg1</td>
<td>GRb1</td>
<td>NGR1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>20.986</td>
<td>138.260</td>
<td>2.750.225</td>
<td>591.103</td>
</tr>
<tr>
<td>60</td>
<td>272.115</td>
<td>5.435.856</td>
<td>7.305.430</td>
<td>2.347.674</td>
</tr>
<tr>
<td>90</td>
<td>73.463</td>
<td>359.755</td>
<td>9.549.556</td>
<td>4.137.341</td>
</tr>
</tbody>
</table>

Fig. 6  The in vivo and in vitro correlation curves for NGR1, GRg1 and GRb1 in bio-adhesive pellets with chitosan. Noting: Q(%) represents the cumulative rates of in vitro release, R represents Fraction Input.

combination were both 3–4 times larger than the control group (pellets without bio-adhesive materials) [21]. The results demonstrated our prepared pellets with bio-adhesive materials had mucous membrane adhesion characteristics.

In the present study, we prepared three types of bio-adhesive pellets, i.e., HPMC, chitosan and chitosan: carbomer (1 : 1), selecting the best preparation for future studies. Because PNS are not stable under acid conditions and degraded easily, choosing the artificial intestinal fluid as release medium to explore drug release behavior is proper. Our results demonstrated that bio-adhesive pellets with HPMC released in sustained fashion for 12 h. Bio-adhesive pellets with chitosan: carbomer (1 : 1) could release completely within 3–4 h. Bio-adhesive pellets with chitosan disintegrated rapidly and released completely, which showed no difference from that normal pellets.

HPMC is a kind of hydrophilic gel material. The bio-adhesive with HPMC has hydrophilic gel skeleton type release mechanism. When HPMC is placed in aqueous medium, due to the hydration and a thick gel barrier formation, internal medicine would slowly spread to the surface and dissolve in medium, which would prolong the time of drug release and block PNS release in the stomach, avoiding PNS degradation in the stomach. The impetus of drugs diffusion comes from drug concentration gradient in the skeleton, and the release behavior is slow after the first fast release. The release mechanism approximated Higuchi equation or first order kinetic equation.

Under high pH conditions, the chitosan expands slowly, gel layer is formed slowly, the solution could seep into the inside of skeleton and dissolve drugs, and eventually the skeleton is disintegrated, leading to the rapid release. Carbomer is different from chitosan; due to its low pKa value, carbomer expands rapidly under the high pH conditions, delaying the release of drugs.

In establishing and validating the analytical methods for determination of in vivo samples, we showed that HPLC-MS/MS could determine three major components NGR1, GRg1, and GRb1 in PNS preparations. We found the NGR1, GRg1, GRb1 of [M−H]− and internal standard of [M+HCOO]− peaks in the negative ion mode with ESI ion source, but the abundance values were not high. On the contrary, the abundance values of [M+Na]+ in the positive ion detection mode with ESI were higher, and the ion pair was stable, so MRM detection of drugs was chosen in positive ion mode [22-23].

During establishing the biological sample pretreatment methods, we investigated the influence of extraction solvents (methylene chloride, methanol and acetone, acetone and methanol), the volume of solvent (2, 3, and 10 times), extraction times (once or twice), and mobile phases (methanol, acetonitrile, formic acid aqueous solution, and ammonium acetate solution) as well as the internal standard. Using methanol or acetonitrile alone as solvent extraction, the extraction efficiency was incomplete, and the matrix effect exceeded the requirement range. After methanol was added into acetone, the ingredients in plasma that were bound to plasma proteins were extracted, further improving the extraction efficiency [24-25]. Therefore, according to the literature [24], the final extraction methods were established as follows: solvent was acetone: methanol (3 : 1), the extraction solvent volume was three times of the amount of samples, and extracting 1 times. The impurities were removed relatively completely, the process was simple and easy to handle, and
the extraction rate and the matrix effect could meet the requirements for the determination of biological samples.

To determine three main ingredients of PNS, NGR1, GRG1, and GRB1, simultaneously, the linear gradient elution method was used in the present study. In HPLC-UV detection, if mobile phase was methanol, the baseline fluctuated widely, and methanol had end absorption which could affect the PNS determination results. But methanol would not affect the function of the mass spectrometry detector with HPLC-MS/MS. So we chose methanol as organic mobile phase. We found that water with ammonium acetate and n-butyl amine solution could significantly improve the efficiency of ionization of three ingredients [26], and therefore, we selected 10 mmol·L^{-1} of ammonium acetate solution (containing 0.5% n-butyl amine) as the water phase.

Internal standard must have similar polarity to NGR1, GRB1 and GRG1 and no obvious difference from their molecular weight, and do not interfere with their determination. According to the relevant literatures [27-30], we chose Digoxin as the internal standard, considering that its polarity, molecular weight (779.5) and retention time were similar to that of the three compounds, which met the requirements for sample analysis.

Our results indicated that the bio-adhesive pellets with HPMC and chitosan : carbomer (1 : 1) had good sustained release effect, the $T_{max}$ values of each ingredient showed different degrees of extension, and the $C_{max}$ values were greater than that of the normal pellets. Bio-adhesive pellets has a quick release or accelerated absorption in vivo process, which was effective absorption enhancer. According to the dissolution of the pellets in the artificial intestinal fluid, the release of bio-adhesive pellets with HPMC and chitosan: carbomer (1 : 1) was slow, and therefore, the $C_{max}$ increases of the bio-adhesive pellets with HPMC and chitosan: carbomer (1 : 1) were due to the acceleration PNS absorption and the extension of $T_{max}$ was the result of sustained release.

Carbomer could open tight junction and promote absorption by paracellular route. Besides, it also has effect of tissue adhesion and enzymatic degradation of gastrointestinal tract. For pellets with chitosan alone, cationic chitosan can interact with anionic membranes, which could open tight junction and promote absorption by paracellular route [31], so as to improve oral bioavailability of the drug.

The three ingredients of PNS analyzed in the present study are water-soluble, and the poor membrane permeability and bile drainage are the two main reasons for low bioavailability. In addition GRG1 undergoes a rapid kidney metabolism [32]. Therefore, delaying the drug release and slowing kidney metabolism could increase oral bioavailability greatly. Indeed, the extent of oral bioavailability increase in GRG1 for all kinds of bio-adhesive materials was the largest among the three ingredients of PNS.

The deconvolution method does not rely on the simulation of compartmental model and is especially suitable for drugs that do not comply with compartmental model and may be suitable for all kinds of in vivo and in vitro correlation research, with the characteristics of simple concept and intuitive mathematical operations, which has been recorded by the United States Pharmacopoeia.

The in vitro release pattern of bio-adhesive pellets with HPMC is through multi-mechanism of diffusion and skeleton dissolution, releasing slowly. In the present study, we got a good correlation between in vivo and in vitro results using the deconvolution method. However, the three ingredients of bio-adhesive pellets with chitosan : carbomer and chitosan showed no significant in vivo and in vitro correlations. Chitosan has different release characteristics under artificial gastric fluid and intestinal fluid conditions. The main factors affecting dissolution of soluble drugs from expansible skeleton are associated with the speed of gel layer formation on the preparation surface. If the gel layer forms slowly, the solution could seep into the inside of skeleton, dissolving drugs, disintegrating skeleton eventually, and causing rapid drug release. Chitosan has good solubility in the artificial gastric fluid and poor solubility in artificial intestinal fluid. Under low pH conditions, chitosan expands rapidly, and the gel layer forms fast, which could delay the release of drugs. Under high pH conditions, drugs release fast, and the drug release behavior reflects drug absorption in vivo.

**Conclusion**

Compared with ordinary pellets, the order of increases in oral bioavailability of bio-adhesive pellets with different bio-adhesive materials were as follows: HPMC pellets > chitosan: carbomer pellets > chitosan pellets > ordinary pellets, which provided further evidence that the preparation of PNS bio-adhesive pellets could improve PNS oral bioavailability. According to previous research [18], HPMC K4M skeleton materials could promote PNS absorption in intestinal perfusion model, and bio-adhesive pellets with HPMC had the characteristics of sustained release and higher bio-adhesive property. Compared with other preparations, the increase in oral bioavailability of PNS bio-adhesive pellets with HPMC was the largest, with a good correlation between in vitro release and in vivo bioavailability, demonstrating that the in vitro release behaviors could forecast the in vivo absorption properties, so as to guide preparation of prescription and technology screening.

**References**


