

Pharmacokinetics and vasodilating effect study of beraprost sodium in healthy volunteers

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Abstract: Currently beraprost sodium (BPS) is widely proposed to ameliorate the symptoms caused by chronic arterial occlusive disease. The objective of this study is to investigate the BPS pharmacokinetic characteristics, its vasodilating effect and the relationship between plasma concentration vs response effect. 12 healthy Chinese volunteers (6 male, 6 female) were chosen to participate in a single center, random, and open design study. After overnight fasting, BPS (dose = 40 μ g) was administrated orally to each volunteer. The blood samples were collected at different time points (from 0 to 5 h after administration) and BPS concentration was analyzed by LC-MS/MS method. The vasodilating effect was evaluated by detecting the skin microcirculation blood flow of volunteers' fingers with laser Doppler fluxmetry. The C_{max} of BPS was (601.14 $\pm$ 214.81) pg/mL, the T_{max} was (0.58 $\pm$ 0.48) h, and AUC_{0-t} was (1020.41 $\pm$ 214.63) pg/mL·h. BPS exhibited significant vasodilating effect since the skin microcirculation blood flow increased definitely at 0.25, 0.5, and 0.75 h (all $p < 0.05$) after drug administration, and a positive correlation was presented between the pharmacokinetics and the vasodilating effect, which would be beneficial for guiding BPS dosage in clinical.

Keywords: Beraprost sodium; pharmacokinetics; vasodilating effect; skin microcirculation blood flow.

INTRODUCTION

Beraprost is the first synthetic stable analogue of prostaglandin I₂ (PGI₂), in which the enol ether moiety has been replaced by a benzofuran ether function. This modification has increased the plasma half-life time from 30 s to several hours and permitted the compound can be dosed orally as its sodium salt, BPS (Nishio *et al.*, 1989). The pharmacokinetic characteristics studies of BPS indicate that BPS is rapidly absorbed after oral administration, with a T_{max} approximately 0.5~1.5 h (Demolis *et al.*, 1993; Lee *et al.*, 2007). BPS is developed as an active PGI₂ analogue with anti-platelet aggregation and vasodilating properties by Toray Industries, Inc (Ohno *et al.*, 1985). The generic drug BPS produced by Beijing Tide Pharmaceutical Co., Ltd. has been approved by CFDA since 2008. At present BPS is widely used to ameliorate chronic peripheral arterial occlusion induced symptoms (ischemic leg ulcers, intermittent claudication, limb pain and a sensation of coolness in the limbs) in clinical (Guan *et al.*, 2003).

Peripheral arterial occlusive disease (PAOD) is an ischemic disease caused by peripheral arteriosclerosis-induced arterial stenosis or occlusion, which results in a lack of adequate arterial blood to the limbs. It is a progressive disease with significant elevated cardiovascular morbidity, mortality and decreased quality of life (Lawall *et al.*, 2016). Patients suffer from diabetes mellitus, hypertension, and lipid metabolism disorder more commonly develop symptomatic PAOD (Atturu *et*

al., 2014). The rapid developed endovascular techniques and drugs are beneficial for the treatment of PAOD, *e.g.*, prostaglandin and prostacyclin have been used to treat critical limb ischemia, especially for patients not suitable for surgical or endovascular revascularization (Atturu *et al.*, 2014). Intravenous PGI₂ can improve total skin blood flow significantly in healthy volunteers (Wollersheim and Thien, 1988), and BPS is effective to improve various subjective symptoms in patients with PAOD (Guan *et al.*, 2003; Yoon *et al.*, 2013). However, although the correlation between pharmacokinetics and platelet antiaggregating effect has been reported (Demolis *et al.*, 1993), few clinical studies have yet described the relationship between the pharmacokinetics and the vasodilating property of BPS. In order to provide more data support for understanding the vasodilating pharmacology mechanism of BPS, we decided to determine the disposition and the skin microcirculation blood flow of BPS in healthy volunteers after single oral administration of BPS to find out the relationship between plasma exposure and response effect.

MATERIALS AND METHODS

Study design

12 healthy Chinese adult volunteers were orally administered single-dosed 40 μ g of BPS on an empty stomach, using a single center, random and open study design. The trial was approved by Ethics Committee of Qilu Hospital, Shandong University according to Declaration of Helsinki and the guidelines of Good Clinical Practice (GCP) (Approval No. KYLL-2016-374).

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Volunteers

12 healthy volunteers [6 male, 6 female; age 26 ± 3 years (mean $\pm$ standard deviation); range 21~31 years; body weight: 169.6 ± 9.2 kg, range 153~182 kg] were recruited via an open recruitment advertisement in the study. All gave their written informed constant to participate. The subjects met the inclusion criterion after the laboratory and medical tests, including height, weight, body temperature, blood pressure, heart rate, respiratory rate, 12-lead Electro Cardio Gram (ECG), blood routine test, urine routine test, and urine pregnancy test (female). All the subjects had no pregnancy plans and were voluntary to contracept within the next 6 months. The main exclusion criterion were age <18 years or >35 years; pregnancy (urine pregnancy test at screening and before test session), lactation or menses during the study session; had personal or family history; HBsAg, HCV, treponema pallidum, or HIV antibody positive; use of medication that may interfere with the study drug; chronic or acute physical illness (abnormal physical examination, ECG, or hematological and chemical blood analyses); illicit drug use, tobacco smoking more than 5 cigarettes/day within the previous 3 months and during the study; take chocolate, drink caffeine or xanthine-containing liquids within 48 hours prior the study. No other medications aside from BPS were used during this study.

Drug administration

All the volunteers were admitted in the trial ward in Institute of Clinical Pharmacy, Qilu Hospital of Shandong University one day in advance. After an overnight fast (9 h), 40 μ g of BPS tablet (Beijing Tide Pharmaceutical Co., LTD, China) was orally administered with 200mL of warm water. The volunteers were required to sit down and keep quiet during the study. Water and standard meals were provided at 2h and 4h after administration, respectively, and no other drink or food was allowed. The experimental environment was kept at $22 \pm 2^\circ\text{C}$ and 40%-50% humidity. The volunteers were allowed to leave the ward next day.

Blood sample collection

Cubital venous blood (5 mL) was collected into EDTA-anticoagulant tubes before (0) and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after BPS administration. The blood samples were immediately centrifuged (3000 g, 10 min, 4°C), and the plasmas were rapidly stored at -80°C until analysis. All the blood samples were collected and processed away from light in ice bath condition.

Finger skin blood flow detection

Laser Doppler imaging (LDI) is feasible to measure the microcirculation under superficial skin (Stark *et al.*, 2008; Luo *et al.*, 2015). The principle of this technique lies in the Doppler frequency shift of reflected laser light by the skin which correlates with the movement of red cells, allowing cutaneous perfusion to be detected on a "flux"

scale (Holland AJ *et al.*, 2002). MoorLDI-HIR LDI system (Moor Instruments, Devon, UK) was used to determine the finger skin microcirculation blood perfusion flux. The detection locations were from the proximal interphalangeal joints to the finger tips of index finger, middle finger, ring finger, and little finger. The images of volunteers' right hand were detected by laser Doppler fluxmetry immediately after blood sample collecting at the time point of before (0) and 0.25, 0.5, 0.75, 1, 1.5 and 2 h after BPS administration.

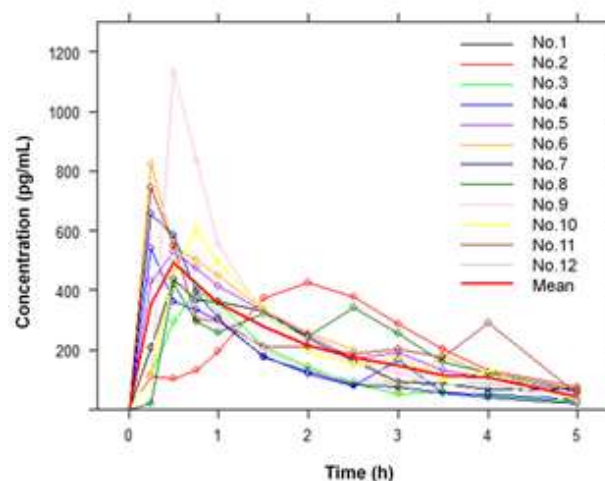


Fig. 1: The plasma concentration-time profiles of 12 volunteers after administration of BPS tablet and the mean C-T curve ($n=12$).

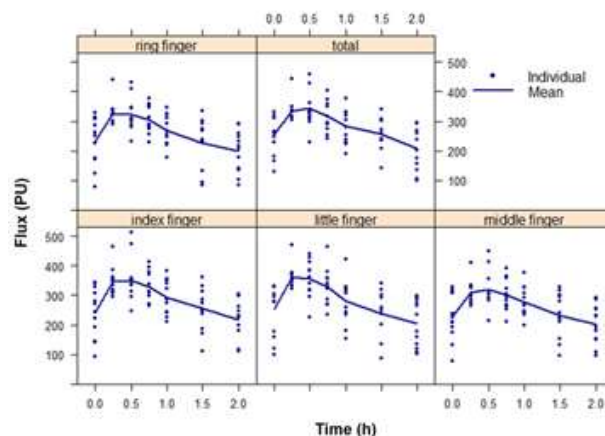


Fig. 2: The mean finger skin microcirculation perfusion flux versus time curves of total four fingers and each finger ($n=12$).

Safety evaluation

Body temperature, blood pressure, and heart rate were assessed repeatedly before (0) and 3, 6, and 24 h after drug administration. The other medical tests were examined before the subjects left the ward. Any adverse events occurred during the clinical study would be recorded carefully.

STATISTICAL ANALYSIS

The pharmacokinetic parameters of BPS were calculated by DAS 2.0 software, including AUC_{0-t} , AUC_{inf} , MRT_{0-t} , MRT_{inf} , $t_{1/2}$, V_z/F , CL/F , T_{max} , and C_{max} . The finger skin microcirculation flow was evaluated by moorLDI Image Review Version 5.3 software, and mean perfusion flux of total four fingers and each fig. were analyzed respectively. All the data were expressed as mean $\pm$ SD. The statistical significance between various groups was analyzed by Student's *t*-test one-way ANOVA. A value of $p < 0.05$ was considered to be statistically significant. Statistical analysis was done using SPSS 17.0 software.

RESULTS

Pharmacokinetics

BPS concentration in plasma was determined using a sensitive and validated LC-MS/MS method according to the reports published previously (Lee *et al.*, 2007; Zhang *et al.*, 2013). Briefly, BPS and indomethacin as an internal standard (IS) in human plasma were extracted through liquid-liquid extraction and separated on a Diamonsil C18 column. The calibration curve of the validated method was linear over the range of 10-1500 pg/mL, with a lowest limit of quantitation of 10 pg/mL, and the intra- and inter-day precision and accuracy were both $< \pm 15\%$. Total 144 blood samples were determined by this validated LC-MS/MS method. The plasma concentration-time (C-T) profiles after administration of BPS tablet of 12 volunteers and the mean C-T curve were

shown in fig. 1. All the C-T curves can be subjected to normal distribution. The C_{max} of orally administrated BPS was (601.14 ± 214.81) pg/mL, and the T_{max} in most volunteers were in the range of 0.25~0.75 h, except for No.2 volunteer, at 2 h after administration. The C_{max} of No.9 volunteer (1133 pg/mL) was much higher than the total C_{max} , which may be caused by the large individual difference. Pharmacokinetic parameters were estimated using a two-compartment model without lag time. The main pharmacokinetic parameters were shown in table 1.

Vasodilating effect

The vasodilating effect of BPS on finger skin microcirculation was estimated by calculating the mean perfusion flux of total four fingers and each one, separately, and the results were shown in table 2. BPS exhibited significant vasodilating effect on finger microcirculation of total four fingers at 0.25 h and 0.5 h after administration compared with 0 h of (all $p < 0.01$). The mean flux-time curves of 12 volunteers were depicted in fig. 2. The mean flux of total four fingers peaked at 0.5 h after administration, that was (333.3 ± 60.5) PU. Time to the maximum flux of ring and little fingers were both at 0.25 h, while that of index and middle finger were at 0.5 h. This time was consistent with the T_{max} (0.58 ± 0.48) h of BPS. The results indicated that the vasodilating property was coincident with the increase of plasma drug concentration in healthy volunteers, and the effect was significant but transient, lasting < 1.5 h. It was interesting to find that the microcirculation blood flux at 2 h after administration was lower than that of 0 h, which may be

Table 1: The main pharmacokinetic parameters of BPS ($n=12$).

Parameters	mean $\pm$ SD
AUC_{0-t} (pg/mL·h)	1020.41 ± 214.63
AUC_{inf} (pg/mL·h)	1192.20 ± 470.52
MRT_{0-t} (h)	1.73 ± 0.30
MRT_{inf} (h)	2.31 ± 0.96
$t_{1/2}$ (h)	1.29 ± 0.43
V_z/F (L)	67.86 ± 30.88
CL/F (L/h)	37.04 ± 10.56
T_{max} (h)	0.58 ± 0.48
C_{max} (pg/mL)	601.14 ± 214.81

Table 2: The mean perfusion flux of total four fingers and each one of 12 volunteers after administration of BPS ($n=12$).

Finger	Flux (PU)						
	0 h	0.25 h	0.5 h	0.75 h	1 h	1.5 h	2 h
Index	242.2 ± 84.8	$345.3 \pm 45.3^{**}$	$348.8 \pm 74.0^{**}$	$327.0 \pm 51.1^*$	293.6 ± 56.6	255.0 ± 71.2	216.2 ± 72.6
Middle	227.4 ± 78.6	$311.2 \pm 39.3^{**}$	$320.3 \pm 61.0^{**}$	$299.8 \pm 52.0^*$	276.8 ± 50.3	232.9 ± 75.4	202.5 ± 66.5
Ring	231.6 ± 78.7	$326.2 \pm 40.8^{**}$	$322.3 \pm 54.7^{**}$	$304.4 \pm 45.9^*$	267.9 ± 52.0	231.2 ± 83.8	199.3 ± 70.9
Little	252.9 ± 87.6	$362.9 \pm 37.9^{**}$	$355.8 \pm 60.8^{**}$	328.1 ± 60.5	279.7 ± 73.2	237.4 ± 91.4	204.8 ± 77.8
Total	236.9 ± 80.0	$332.7 \pm 38.2^{**}$	$333.3 \pm 60.5^{**}$	$312.6 \pm 50.0^*$	278.5 ± 55.3	238.2 ± 76.7	205.3 ± 70.0

$^{**}p < 0.01$, compared with each 0 h group; $^*p < 0.05$, compared with each 0 h group.

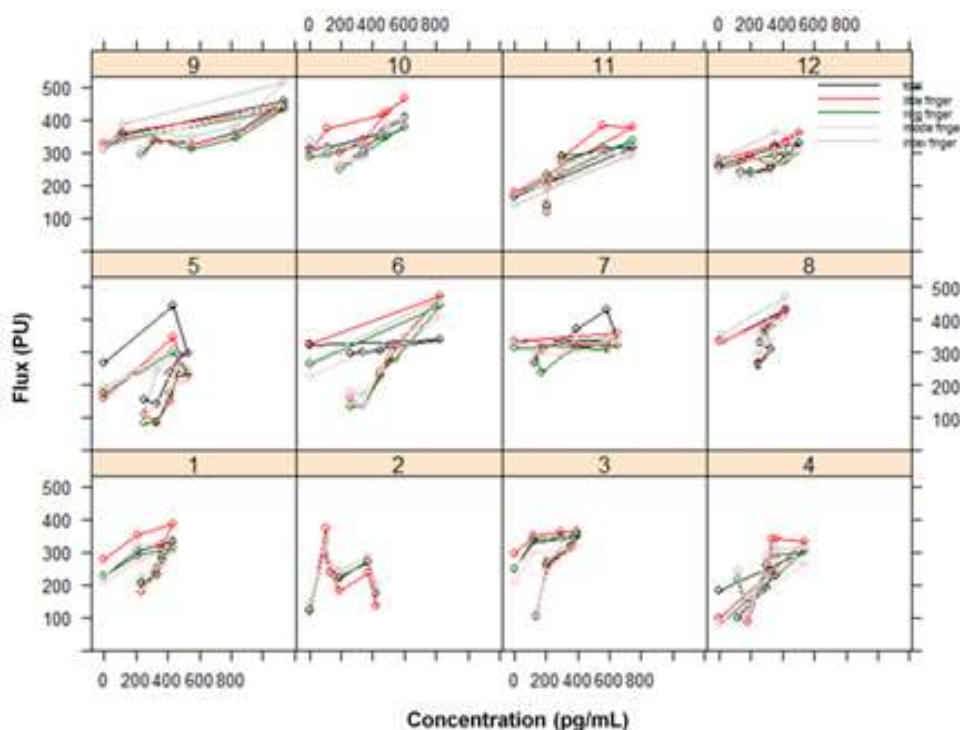


Fig. 3: The plasma concentration-effect plot of orally administrated BPS of total four fingers and each finger in 12 volunteers.

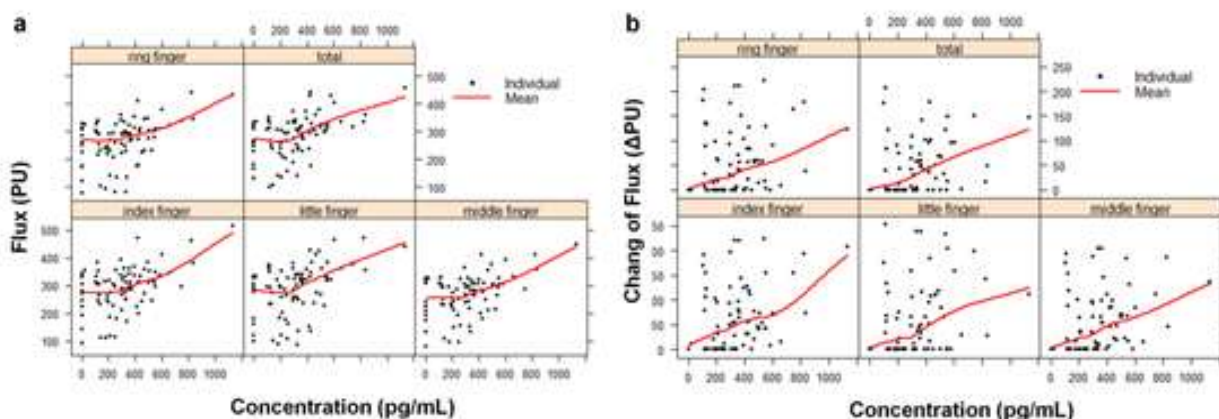


Fig. 4: The profiles of the relationship between plasma concentration and response of BPS. a. The relationship between the concentration of BPS and flux of each finger and mean of total four fingers. b. The relationship between the concentration of BPS and the change of flux of each finger and mean of total four fingers.

due to the regulation by the body's negative feedback in some extent.

Plasma concentration-effect relation

The plasma concentration and vasodilating effect relationship was described by the profile of measured concentration and microcirculation perfusion flux. Fig. 3 showed the plot of microcirculation perfusion flux *versus* plasma concentration exhibited clockwise loop of total four fingers as well as each finger of most volunteers. As shown in fig. 4a, the microcirculation perfusion flux and plasma concentration was nearly linear relationship. Fig. 4b showed the relationship between the change of flux

and plasma concentration and they also presented positive linearity, which indicated the pharmacokinetics and vasodilating pharmacology of BPS was in general direct response.

Safety and tolerability

No significant hemodynamic effect or other adverse reaction of BPS was observed during the study after single administration. Only No.10 volunteer appeared facial flushing and dizziness and lasted for 35 min after administration. These effects were transient and disappeared spontaneously and the volunteer did not quit the study.

DISCUSSION

So far, no report has published the relationship between pharmacokinetics and vasodilating property of BPS. The present study investigated the pharmacokinetic characteristics and vasodilating effect after oral administration of BPS 40 μ g in healthy volunteers, and meanwhile explored the plasma concentration-effect relationship. There have been few reports on the pharmacokinetics of BPS, and most studies focused on the pharmacodynamic effects, including platelet-aggregation inhibition (Lee *et al.*, 2007; Nony *et al.*, 1996), diabetic nephropathy remission (Na *et al.*, 2013; Shima *et al.*, 2015), pulmonary arterial hypertension therapy (Sakao *et al.*, 2014), and peripheral circulation insufficiency improvement (Guan *et al.*, 2003; Murai *et al.*, 1989). Through determining the plasma concentration of BPS in 12 healthy Chinese volunteers by LC-MS/MS method, the mainly pharmacokinetic characteristics of BPS were obtained in our study. Results showed that BPS pharmacokinetics was linear and was rapidly absorbed after oral administration, with the mean C_{max} value of 601.14pg/mL reached at 0.58 h. The C_{max} was a little higher than that of observed in the study of Lee and associates (Zhang *et al.*, 2013), who found that C_{max} was 420 pg/mL after a single dose of 60 μ g. This difference may be caused by the established determination method and the less sample size in both studies. The AUC was estimated at (1020.41 $\pm$ 214.63) pg/mL·h for up to 5 h. The terminal half-life of BPS in this study was (1.29 $\pm$ 0.43) h, which was somewhat longer than that of reported ranging from (0.50 $\pm$ 0.21) h to (0.91 $\pm$ 0.27) h independently of BPS dose (Lee *et al.*, 2007), and we speculated that the difference may be due to the different drug production, the different races, as well as the individual variation between volunteers.

The vasodilating effect study of BPS indicated it could increase the finger skin microcirculation blood flow in healthy volunteers and thus providing study basis for its application in PAOD therapy. The mean perfusion flux of total four fingers reached peak value at 0.5 h, which was nearly the same time as the T_{max} of BPS. The vasodilating effect lasted until 1.5 h and then resumed to the initial level or even lower, indicating no drug accumulation after single dose. A clockwise loop relationship appeared between the perfusion flux and BPS concentration, and there was a linear relationship between the flux or the change of flux and the concentration. The results displayed a positive correlation between BPS concentration and the vasodilating effect, like the rapid absorption and elimination characteristics of pharmacokinetics, the effect also showed rapid increase and decrease after drug administration, indicating the pharmacokinetics-pharmacodynamics model of BPS in terms of vasodilating effect was the direct response model. Previous study has reported pharmacokinetics and platelet

antiaggregating effect of BPS in healthy volunteers, and found BPS (40 or 60 μ g) exerted its maximal antiaggregating effects between 0.5~1 h. However, no significant correlation existed between the maximum antiaggregating effect and the corresponding peak plasma concentration of APS-314d, the active component of BPS (Lee *et al.*, 2007), which displayed the vasodilating and the antiaggregating effects were different mechanisms according to pharmacokinetics.

The adverse drug reactions including headache and flush were common after BPS administration (Lee *et al.*, 2007). Only one volunteer was observed to appear transient flush and dizziness in our study, and the adverse effects were obviously attributed to BPS because the maximum intensity of the effects corresponded to the peak concentration at 0.75 h after administration, therefore these side effects were dose dependent. No other adverse drug reactions were observed during the study, and the changes of medical and experimental examination of the volunteers before and after the trial were assessed to be without clinical significance.

CONCLUSION

This single center, random, and open design clinical trial demonstrated that orally administration of 40 μ g BPS exerted transient and significant vasodilating effect in healthy volunteers and this effect was closely related to the drug plasma concentration. A positive correlation was presented between the pharmacokinetics and pharmacodynamics of BPS in this investigation. The time-concentration-perfusion flux relationship study may provide research foundation for deep understanding of the pharmacology mechanism of BPS used to treat PAOD in clinical practice.

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