BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **13**: 442–444 (1999)

ORIGINAL RESEARCH

Relationship between the clinical effects of berberine on severe congestive heart failure and its concentration in plasma studied by HPLC

Xiangji Zeng* and Xianghong Zeng

Chengdu University and Section of Cardiology, Chengdu Second Municipul Hospital, Chengdu, Sichuan 610081, People's Republic of China

Received 23 February 1998; revised 8 September 1998; accepted 16 November 1998

ABSTRACT: It has been reported that berberine is valuable for long-term treatment of ventricular premature beats (VPBs) and leads to a decrease in mortality for patients with congestive heart failure (CHF). In order to improve its therapeutic value and reduce its side effects, it is necessary to study the relationship between its activity and plasma concentration in patients with CHF. Patients with CHF were treated with conventional therapy for 2 weeks. Immediately after the data from a dynamic electrocardiogram (DCG) and left ventricular ejection fraction (LVEF) were obtained, 1.2 g/day of oral berberine was given. After 2 weeks of berberine therapy, the DCG data and LVEF were reassessed and the plasma berberine concentration was measured by HPLC. Plasma samples were pretreated by extraction with chloroform. Berberine in all samples was determined using a μBondapak C_{18} column, a mobile phase of acetonitrile:0.02 mol/L phosphoric acid (45:55, v/v), and a UV detector at 346 nm. The mean recovery was 96.5%. The linear range was 40–1600 ng/mL. The detection limit for berberine in plasma was 0.4 ng. The decrease in frequency and complexity of VPBs and the increase in LVEF in patients with plasma berberine concentrations higher than 0.11 mg/L ($n = 31$, group B) were more significant than at concentrations lower than 0.11 mg/L ($p < 0.01$ vs $p < 0.05$). Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Berberine is an alkaloid widely distributed in nature. Along with its preparations (mainly berberine sulfate and berberine chloride), it has been used intensively by orientals as an intestinal antiseptic by oral administration. Other systematic effects have also been reported, such as hypotensive effects, bilirubin excretion enhancement, inotropic, sedative and anti-inflammatory effects, dilation of coronary artery, anticoagulation, slighly lowered heart rate, acceleration of repair of pancreatic beta cells, and lowering of elevated low density lipoprotein cholesterol (LDL-C). In a recent study berberine, being a class III antiarrhythmic drug, was found to be of value for long-term treatment of ventricular premature beats (VPBs) and decreasing mortality for patients with congestive heart failure (CHF) (Zeng *et al.*, 1997). In order to improve its clinical value and reduce its side effects, we have studied the relationship between its activity and concentration in plasma for patients with chronic CHF.

*Correspondence to: X. Zeng, Chengdu University and Section of Cardiology, Chengdu Second Municipal Hospital, Chengdu, Sichuan 610081, People's Republic of China.

Abbreviations used: CHF, congestive heart failure; DCG, dynamic electrocardiogram; LDL-C, low density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; RSD, relative standard deviation; VPBs, ventricular premature beats; VT, ventricular tachycardia.

PATIENTS AND METHODS

Patients. Fifty-six patients with CHF (34 males and 22 females) were studied while admitted to Chengdu Second Municipal Hospital. Their mean age was 62 ± 14 (range 45–76). The patients had severe choronic CHF with New York Heart Association (NYHA) functional classes III ($n = 38$) and IV ($n = 18$), and also had frequent VPBs ($\geq$90/h) and/or ventricular tachycardia (VT). All patients had a reduced left ventricular ejection fraction (LVEF; $21 \pm 8\%$ range 11–27%), assessed by two-dimensional Doppler color echo cardiography. The etiology of chronic congestive heart failure was coronary artery disease in 39 patients and idiopathic dilated cardiomyopathy in the other 17. All patients received treatment with loop diuretics and angiotensin-converting enzyme inhibitors; 51 of them took digoxin and 46 of them nitrates for 2 weeks. None had history of hepatic or renal disease or abnormal hepatic or renal functions.

Electrocardiographic monitoring analyses. All patients went through a three-channel (modified V_5, V_3 and III leads) 24 h Holter electrocardiogram recording (Primmer EP 9900, USA) twice. One recording was obtained after conventional therapy for 2 weeks and just before the berberine therapy, the other recording was taken after berberine administration for 2 weeks. During recording, patients were allowed to perform normal leisure activities. For each 24 h recording, the total and complexitive VPBs were determined and classified by Lown's classification.

Echocardiography. Standard parasternal and apical views were obtained. Images were generated with a Sonos 1500 apparatus

CCC 0269–3879/99/070442–03 $17.50

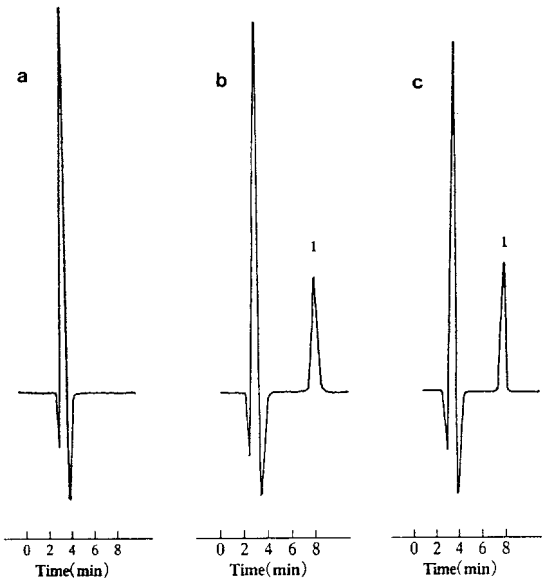

Figure 1. Chromatograms of blank plasma (a), berberine standard in blank plasma (b), and patients' plasma after oral administration of berberine (c); 1, berberine.

(Hewlett-Packard, Andover, MA) with a 2.0/2.5 MHz probe, digitized and then stored in quad-screen cine loop format with a Freeland Cardiac Workstation (Louisville, CO, USA) for the on-line and off-line wall motion analysis. The images taken before and after giving berberine for 2 weeks were recorded. Left ventricular ejection fraction was calculated according to the guidelines of the American Society of Echocardiography.

HPLC DETERMINATION OF BERBERINE IN PLASMA

Drugs and reagents. Berberine tablets were supplied by Southwest Pharmaceutical Ltd Co. (Chongqing, China). The berberine standard was supplied by the Chinese Drug and Biological Product Inspection Institute (Beijing, China). All chemicals and solvents were of either HPLC or analytical grade. Water used in the mobile phase was double distilled.

Instrumentation and chromatographic system. The HPLC system consisted of a Waters liquid chromatograph (Model 510 pump, model 730 data processing system, model u6k injector, Waters, USA) and a model SPD-10A UV–vis detector (Shimadzu, Japan). The column was a μBondapak C_{18} column (10 μm, 300 × 3.9 mm i.d., Waters, USA). The mobile phase was prepared by mixing acetonitrile with 0.02 mol/L phosphoric acid (45:55, v/v). The mobile phase was filtered through a 0.45 μm membrane filter and then degassed. The flow rate of mobile phase was 1 mL/min. The column temperature was kept at 25°C. The chromatogram was monitored using a UV detecter at 346 nm.

Experimental procedure. Immediately after the dynamic electrocardiogram (DCG) and LVEF data were obtained, oral berberine 1.2 g/day was given to the patients. In the morning after 2 weeks of berberine therapy, 2 mL blood was withdrawn 2.4 h after oral administration and an antithrombotic agent was added to it at once. The plasma was prepared by centrifugation.

To eliminate the interference from plasma, a 1.0 ml sample was extracted with chloroform (1 mL × 3). The extracted chloroform phase was dried at 65°C. The residue was dissolved in 1.0 ml methanol, ultrasonicated for 1 min and filtered through a 0.45 μmembrane filter. The filtrate was kept for injection.

Statistics analysis. Values were expressed as mean±standard deviation. Comparisons between two groups were carried out with the Student *t*-test for paired and unpaired data and with the rank sum test for ranked data. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chromatography of berberine

Figure 1 presents a typical chromatogram of berberine in plasma; the retention time of berberine was 8 min. Standard berberine methanol solution samples in various concentrations were injected. The relationship between berberine weight (X) and its corresponding peak height (Y) could be expressed by a linear regression equation $Y = 0.03709 + 6.3354X$ ($r = 0.9998$). The calibration graph for berberine in plasma was linear over the range 40–1600 ng/mL with a 10 μl sample. The detection limit of berberine was 0.4 ng (signal-to-noise, ratio = 2).

To calculate the recovery of berberine, standard berberine methanol solutions were mixed with healthy human blank plasma. Then the peak heights were measured under the same conditions as described above and compared with those of corresponding standard berberine methanol solutions. The mean recovery was 96.5%. The accuracy and precision tests for berberine in plasma were evaluated over the range 49–980 ng/mL. The relative standard deviation (RSD) varied from 2.2 to 4.3%.

Clinical results. The plasma concentrations of berberine in patients with CHF were measured 2.4 h after the oral administration. The results from 56 patients with Lown class 0-V CHF are listed in Table 1.

According to Table 1, all 56 patients with CHF had improved LVEF ($p < 0.05$) and decreased frequency and complexity of VPBs ($P < 0.01$) when receiving 2 weeks of berberine therapy. The subgroup analysis showed that both groups A and B experienced some increase in LVEF, however the increase of LVEF in group B (with plasma berberine concentration 0.19 ± 0.08mg/L) was greater than that in group A (with concentration 0.07 ± 0.04 mg/L; $p < 0.01$ vs $p < 0.05$).

Although there were significant decreases in VPBs frequency in the two groups after berberine treatment, the decrease in group B was more than that in group A

Table 1. Relationship between clinical effects of berberine on ischemic heart failure and its concentration in plasma

	A ($n = 25$) Berberine treatment		B ($n = 31$) Berberine treatment	
	Before	After (mg/L)	Before	After (mg/L)
Concentration of berberine	0	0.07 ± 0.04	0	0.19 ± 0.08[c]
LVEF	21.4 ± 8.1	23.9 ± 6.2[a]	20.7 ± 7.9	27.1 ± 3.4[b,c]
VPBs	2364 ± 1598	1156 ± 736	2408 ± 982	511 ± 616
Lown class				
0	0	1	0	3
I	0	2	0	12
II	4	5	9	9
III	10	8	8	6
IV B	8	7	10	1
V	3	2	4	0

[a] $p < 0.05$;
[b] $p < 0.01$, before vs after treatment of berberine.
[c] $p < 0.05$, A vs B.

($p < 0.05$), especially complexitive VPBs, eg VT in group B decreased more significantly than that in group A ($p < 0.005$).

Therefore, after berberine treatment, the decrease in frequency and complexity of VPBs and the increase in LVEF for patients with plasma berberine concentration of 0.19 ± 0.08 mg/L ($n = 31$, group B) were more significant than those for patients with the concentration 0.07 ± 0.04 mg/L ($n = 25$, group A; $p < 0.01$ vs <0.05). There were no apparent side effects or arrhythmogenesis in all patients.

DISCUSSION

Many thousands of patients with CHF take medications because of a cardiac arrhythmia. There is increasing awareness that approximately 15% of patients show inducing or worsening CHF and arrhythmogenesis, which can be life-threatening, especially at severe CHF with lower LVEF (<30%; Pratt *et al.*, 1989). The results of the Cardiac Arrhythmia Suppression trial study also highlighted the possible dangers of antiarrhythmic drug treatment (Echt, 1991). This is probably related to their negative inotropic effect, inducing coronary artery spasm (Pratt *et al.*, 1989). Many clinical observations have demonstrated that berberine is an antiarrhythmic type III agent. It also has a positive inotropic effect, dilation of cororary arteries and inhibition of alpha adrenergic receptors. In our previous study, it was found that oral berberine might be safe and effective in patients with CHF. However, the pharmacokinetic properties of oral absorption in patients with CHF had hardly been reported. The pharmacokinetic properties of oral absorption of berberine have been reported for healthy volunteers (Li *et al.*, 1995). The concentration of berberine in human blood could reach a level which is high enough to inhibit the transport of potassium and prolong the action (potential duration in the electrophysiological experiment in the guinea pig, Li *et al.*, 1995). For five patients with CHF, after a single oral dose of 300 mg berberine, blood samples were taken at following time intervals: 0.5, 1, 2, 3, 6, 9, 12 and 24 h. A plasma berberine concentration–time curve fitted the one-compartment open model. The T (peak) was 2.39 ± 0.03 h. Therefore, blood samples were withdrawn 2.4 h after oral administration of berberine.

The results showed that the decrease in frequency and complexity of VPBs and the increase in LVEF for patients is related to their plasma berberine concentrations. Therefore, monitoring berberine concentration in plasma is necessary. The method established in this study is satisfactory for separation and measurement of plasma berberine concention. It was convenient, accurate and helpful in treating patients with CHF.

The amount of berberine in plasma was a very small part of that administered. The fate of berberine remains unknown, although metabolism seems to be most likely (Chen *et al.*, 1995). Further investigation is needed.

REFERENCES

Chen, C.-M. and Chang, H.-C. 1995. *J. Chromatogr. B*, **665**:117.
Echt, D. S. 1991. *New Engl. J. Med*, **324**:781.
Li, B. X., Zhang, M. S. and Bao, L. H. 1995. *Harbin Med. Univ. J.* (in Chinese) **29**:382.
Pratt, C. M., Eaton, T., Francs, M., Woolbere, S., Mahamarian, J., Roberts, R. and Young, J. B. 1989. *Am. Heart J.* **118**:433.
Zeng, X. H., Zeng, X. J., Lee, Y. Y. and Huang, Y. H. 1997. *J Heart Failure*, **4**:66.