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Effects of Shigyaku-san (TJ-35) on Pharmacokinetics and Pharmacodynamics of Nicardipine

TSUKASA UNO*1, TADASHI OHKUBO*1, KAZUNOBU SUGAWARA*1, AKIHIRO HIGASHIYAMA*2 and SHIGERU MOTOMURA*2

Department of Pharmacy, Hirosaki University Hospital*1, and Department of Pharmacology, Hirosaki University School of Medicine*2

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The bioavailability of nicardipine has been markedly augmented by grapefruit juice, and this phenomenon has been considered to be due to the effects of naringin, a bioflavonoid found in grapefruit juice. Shigyaku-san (TJ-35), one of oriental herbal medicines (Kampo medicines), contains several bioflavonoids, such as naringin and hesperidin which have been extracted in water from immature oranges and formulated as a galenical preparation. The content of naringin in TJ-35 is 80.27 mg/7.5 g, which is clinically equivalent to the amount of naringin in state the volume of grapefruit juice. In the present study, we describe the effects of TJ-35 on the pharmacokinetics and pharmacodynamics of oral racemic nicardipine.

The pharmacokinetics and pharmacodynamics of (+)- and (−)-nicardipine were studied in four male subjects. The study was conducted as a crossover design after a 4-week wash-out period. Thirty minutes after receiving 7.5 g of TJ-35 with 200 ml of water, each subject was given 40 mg of nicardipine, two 20 mg tablets. Control study was done same procedure without TJ-35. The stereoselective pharmacokinetic parameters (mean±S.D.) of nicardipine were not affected by TJ-35 compared with control treatment of nicardipine: (182.8±110.5 ng•hr/ml with water versus 181.7±50.6 ng•hr/ml with TJ-35 for the area under the concentration-time curve (AUC) and 87.2±19.5 ng/ml with water versus 88.0±44.9 ng/ml with TJ-35 for the peak plasma concentration (C_{max}) of (+)-nicardipine, 62.5±19.5 ng•hr/ml with water versus 83.7±72.8 ng•hr/ml with TJ-35 for AUC and 26.4±11.0 ng/ml with water versus 38.0±27.7 ng/ml with TJ-35 for C_{max} of (−)-nicardipine). Pharmacodynamic parameters of nicardipine such as blood pressure, heart rate and electrocardiograms were not affected by TJ-35.

These results suggest that the factor in grapefruit juice that is responsible for increasing the bioavailability of nicardipine is something other than naringin in TJ-35 and that

*1 〒036-8563　弘前市本町53: 53 Honcho, Hirosaki 036-8563, Japan
*2 〒036-8562　弘前市在府町5: 5 Zaifucho, Hirosaki 036-8562, Japan

factors other than naringin in grapefruit juice may be important for CYP3A4 inhibiting activity.

Key words —— Shigyaku-san, nicardipine, naringin, pharmacokinetics, pharmacodynamics

Introduction

Nicardipine hydrochloride, a chiral 1,4-dihydropyridine derivative is a commonly used orally-administered calcium channel-blocking agent with potent vasodilating activity[1]. It is used for the treatment of hypertension[2] and cerebrovascular disease[3]. The systemic bioavailability of racemic nicardipine is low, less than 30%, despite its almost complete gastrointestinal absorption[4,5]. This is pharmacologically caused by extensive presystemic biotransformation to an inactive primary pyridine metabolite by enzymes of the cytochrome P-450 (CYP)3A4[4]. Nicardipine is a racemic mixture of equal amounts of (+)- and (−)-nicardipine. The pharmacological potency of the (+)-enantiomer of nicardipine has been found to be three times higher than that of the (−)-enantiomer[6].

Recently, grapefruit juice has been reported to augment the oral bioavailability of the dihydropyridine calcium antagonists, including: nifedipine[7], felodipine[7], nitrendipine[8] and nisoldipine[9]. In previous papers, we reported that grapefruit juice enhanced the bioavailability of nicardipine[10] and manidipine[11]. These previous papers suggested that the mechanism probably involved inhibition of presystemic dihydropyridine metabolism at the initial oxidative and subsequent metabolic step[12]. Subsequent papers suggested that bioflavonoids in grapefruit juice could extensively inhibit the first-pass metabolism of these dihydropyridines by CYP3A4[12]. The effects of these flavonoids on felodipine and nifedipine oxidation by human liver microsome has been studied by Guengerich and Kim[13] and Miniscalco et al.[14]. Naringin, one of the compounds considered to be a possible inhibitor, is the most prevalent bioflavonoid in grapefruit juice[15].

Shigyaku-san (TJ-35) is a traditional oriental herbal medicine (Kampo medicine) that has been widely used for oriental anti-ulcer herbal preparations in Japan as well as in China. TJ-35 has various biological activities such as improving mucosal blood flow, promoting mucous secretions, and inhibition of gastric secretion[16]. TJ-35 contains several bioflavonoids that have been derived from immature oranges, hesperidin and naringin in water extraction, and which are present in the galenical form.

Based upon the results of previous studies[12] that described the bioavailability enhancing effects by naringin, we speculated that concomitant oral administration of TJ-35 and nicardipine might increase the bioavailability of nicardipine. In the present paper, we describe the effects of TJ-35 on the pharmacokinetics and pharmacodynamics of oral racemic nicardipine in healthy male subjects.

Materials and Methods

Materials and Reagents

Nicardipine was purchased from Shigma Chemical Co. (St. Luis, MO, USA). Barnidipine as an internal standard as nicardipine

determination in high-performance liquid chromatography (HPLC) method was kindly donated by Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan). Nicardipine tablet (20 mg of Perdipine® tablet bland of nicardipine, Yamanouchi) was purchased from Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan). TJ-35, powder of extracts (Shigyaku-san) was obtained from Tsumura & Co. Ltd. (Tokyo, Japan). Sep-pak® C_{18} cartridge was purchased from Waters Co. (Bedford, MA, USA). All other solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals of analytical grade were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan).

Subjects

Four healthy male subjects (mean age± S.D.: 34.5±7.6 years, mean body weight±S.D.: 61.8±5.1 kg) participated in this study. Protocol was approved by the Ethics Committee of Hirosaki University Hospital, and all subjects gave written informed consent. All subjects were healthy as judged by medical history. Subjects refrained from alcohol and medications, including over the counter drugs throughout the study.

Protocol

The study was conducted as a crossover design after a 4-week wash-out period. Thirty minutes after receiving 7.5 g of TJ-35 with 200 ml of water, each subject was given 40 mg of nicardipine, two 20 mg tablets. Control study was done same procedure without TJ-35. The amount of naringin in TJ-35 was 80.27 mg/7.5 g, which is clinically equivalent to the amount of naringin in state the volume of grapefruit juice[15], and it had been quantitated by the HPLC methods[17] previously described.

Blood samples (10 ml) were collected by venipuncture before the dose and at 0.5, 1, 2, 3, 4, 6 and 8 hours after an oral nicardipine dose. No food was allowed until 3 hours after drug administration. At the same times as blood samplings, blood pressure, heart rate and electrocardiograms were measured.

Assay

Plasma (+)- and (−)-nicardipine concentrations were measured in duplicate by the HPLC method previously described[18]. (+)-Barnidipine (100 ng) in 10 μl methanol was added to the 1 ml plasma sample as an internal standard. The plasma sample was diluted with 5 ml of 1 M/L sodium chloride solution and the solution was briefly mixed. The mixture was applied to a Sep-pak® C_{18} cartridge. The cartridge was then washed with 10 ml of water and 10 ml of 40% methanol. The fraction desired was eluted with 5 ml of 80% methanol. The eluate was evaporated to dryness in vacuum at 60°C. The residue was dissolved in 0.2 ml of chloroform, and the sample was injected onto the HPLC system. The HPLC system consisted of a Rheodyne Model 7120 injector (Rheodyne Inc., Cotani, Calif.), and another Rheodyne Model 7120 injector was used as a switching valve. Precolumn (10 mm×4.6 mm internal diameter) packed with trimetylsilirated silica stationary phase (5 μm) was prepared in our laboratory. The analytical column contained Sumichiral OA-4500 stationary phase (5 μm, 250 mm×4.6 mm internal diameter) (Sumica Chemical Analysis Service, Ltd., Osaka, Japan). A Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) and Jasco Uvidec 980 ultraviolet detector (Jasco, Tokyo, Japan) were used. The wave length was set at 254 nm. The mobile phase consisted of n-hexane-1,2-dichloroethane-

methanol-trifluoroacetic acid (250 : 140 : 10 : 1, vol/vol), and the flow rate was 1 ml/min at ambient temperature.

Data analysis

The area under the concentration-time curve of (+)- and (−)-nicardipine from 0 to 8 hours (AUC_{0-8}) was calculated by the trapezoidal rules. Similarly, the area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$), or total AUC, were calculated from $AUC_{0-8}+C_8/Ke$, in which C_8 is the plasma concentration of nicardipine enatiomers at 8 hours after dosing. The elimination rate constant (Ke) of nicardipine enantiomers was estimated from the nonlinear least-squares regression analysis of the terminal log-linear concentration-time data, and the elimination half-life ($t_{1/2}$) was calculated from 0.693/Ke. The peak plasma concentration (C_{max}) and the time reached to C_{max} (T_{max}) were read from the observed plasma concentration-time data in each of the individuals and used to eliminate the rate of drug absorption. The apparent total clearance (CL_{tot}) was calculated as follows: Dose/total AUC.

Differences in pharmacokinetic parameters for each of nicardipine enatiomers and those after coadministration of TJ-35 were analyzed by the paired Student's t test and $P<0.05$ was considered statistically significant.

Results

Mean time courses for (+)- and (−)-nicardipine concentration in plasma before and after TJ-35 administration are shown in Fig. 1 and Fig. 2, respectively. Pharmacokinetic parameters of (+)- and (−)-nicardipine before and after the administration of TJ-35 are summar-

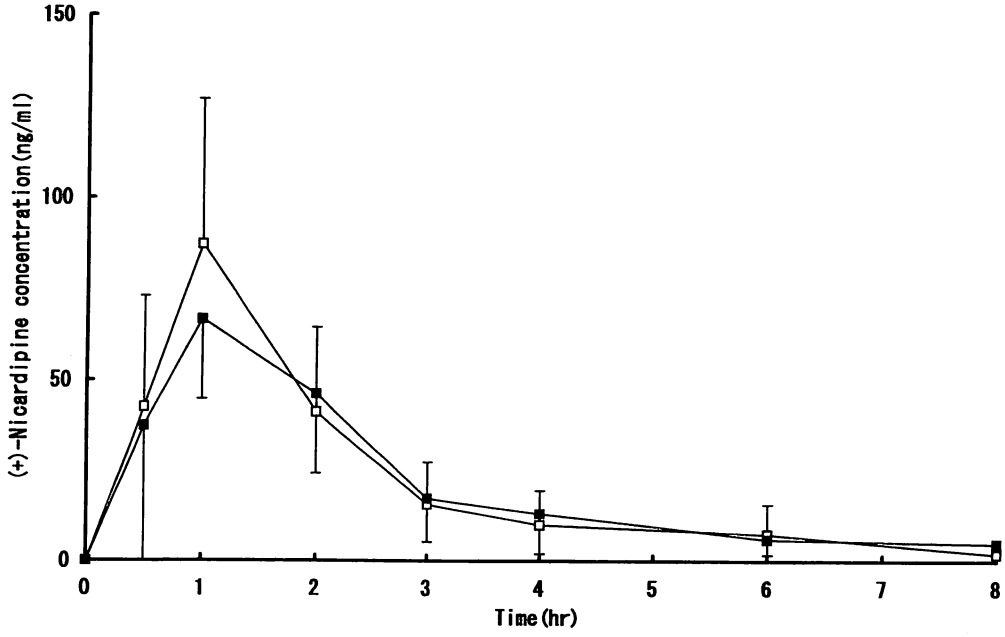

Fig. 1. Effects of TJ-35 (Shigyaku-san) 7.5 g (p.o.) on (+)-nicardipine plasma concentration after the administration of nicardipine 40 mg (p.o.) in 4 healthy volunteers.

ized in Table 1. No significant changes were observed on pharmacokinetic parameters of (+)-nicardipine including $AUC_{(0-8)}$ (175.1±105.9 ng•hr/ml versus 167.3±42.0 ng•hr/ml), Ke (0.35±0.11 hr^{-1} versus 0.27±0.15 hr^{-1}) and CL_{tot} (32.2±13.6 ml/min/kg versus 40.9±26.2 ml/min/kg) between before and after TJ-35. Similarly, parameters of (−)-nicardipine for before and after TJ-35 did not change in this experiment (Table 1). Pharmacodynamic assessments of arterial blood pressure and heart rate on time courses after dosing are shown in Fig. 3 and Fig. 4. TJ-35 did not alter any assessed pharmacodynamic parameters compared with water in this study.

Discussion

In our previous results, grapefruit juice caused a 1.4-fold and 1.8-fold increase in $AUC_{0-\infty}$ of (+)- and (−)-nicardipine after oral administration, respectively[10]. Associated with this increase, grapefruit juice produced marked effects on heart rates at 1 and 2 hours after oral administration compared with water. In the present results, pharmacokinetic parameters of nicardipine did not significantly change after the treatment of TJ-35. This suggests that high content of naringin in TJ-35 did not inhibit CYP3A4 activity. These results indicate that naringin in grapefruit juice does not account for the increase of nicardipine bioavailability. Bailey et al. has described that the coadministration of naringin with nisoldipine did not change nisoldipine pharmacokinetics in human[9].

Recently, Edwards et al. has reported that the dilution of grapefruit juice with a naringin solution reduced the inhibitory activity of

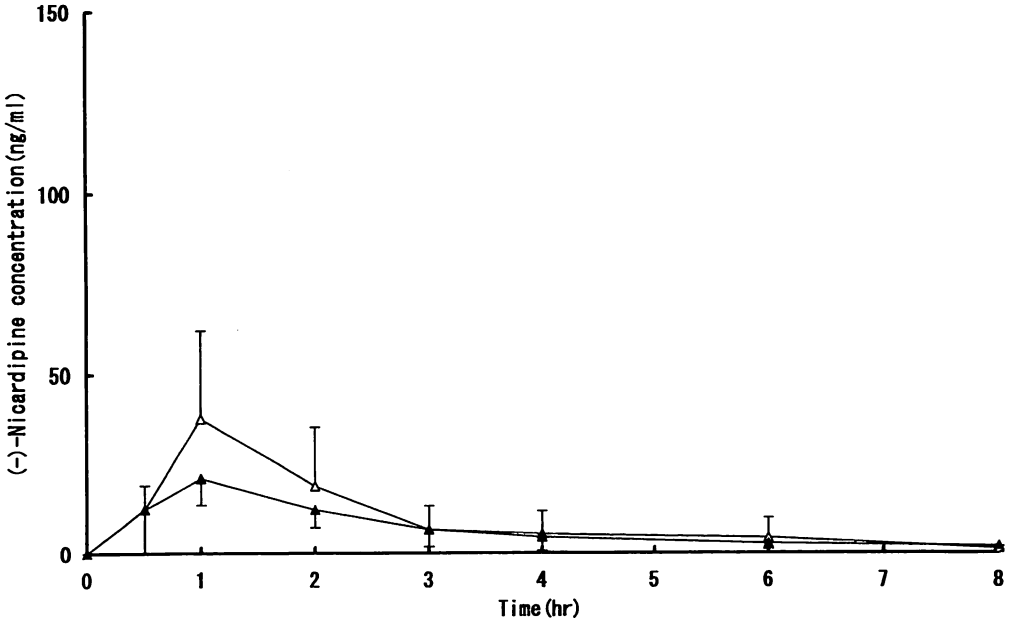

Fig. 2. Effects of TJ-35 (Shigyaku-san) 7.5 g (p.o.) on (−)-nicardipine plasma concentration after the administration of nicardipine 40 mg (p.o.) in 4 healthy volunteers.

Table 1. Pharmacokinetic parameters of nicardipine enantiomers after an oral racemic 40 mg dose and those following pretreatment with TJ-35 (Shigyaku-san) 7.5 g in 4 healthy volunteers

Parameters	(+)-Nicardipine		(−)-Nicardipine	
	Control	with TJ-35	Control	with TJ-35
AUC $_{0-8}$ (ng•hr/ml)	175.1±105.9	167.3±42.0	78.5±72.8	55.3±16.8
AUC $_{0-\infty}$ (ng•hr/ml)	182.8±110.5	181.7±50.6	83.7±72.8	62.5±20.4
Ke (hr^{-1})	0.35±0.11	0.27±0.15	0.27±0.15	0.23±0.03
$T_{1/2}$ (hr)	2.2±0.9	2.6±1.1	3.3±1.9	3.1±0.4
C_{max} (ng/ml)	88.0±44.9	87.2±19.5	38.0±27.7	26.4±11.0
T_{max} (hr)	0.9±0.3	0.9±0.3	0.9±0.3	0.9±0.3
CL_{tot} (ml/min/kg)	32.2±13.6	40.9±26.2	95.2±16.8	107.9±79.5

Data are mean values±S.D.

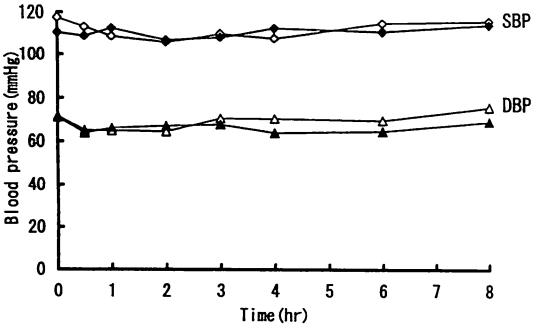

Fig. 3. Mean of changes in systolic and diastolic blood pressure (SBP and DBP) after nicardipine 40 mg oral dose (◇ and △) and with TJ-35 (Shigyaku-san) (◆ and ▲) in 4 healthy volunteers.

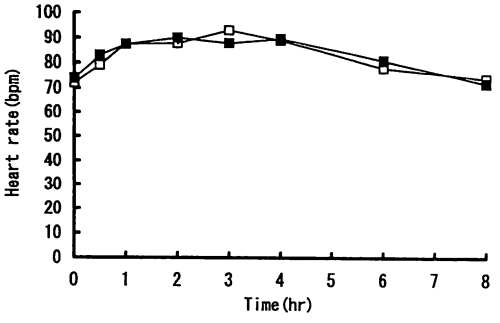

Fig. 4. Mean of changes in heart rate after nicardipine 40 mg oral dose (□) and with TJ-35 (Shigyaku-san) (■) in 4 healthy volunteers.

grapefruit juice even though naringin concentration did not change[19]. These results are consistent with our observations. In a recent report, the content of CYP3A4 in small intestine was decreased by intake of grapefruit juice, without change of liver CYP3A4[20]. These finding suggest that grapefruit juice may be a selective inhibitor for intestinal CYP3A4. It may be concluded, therefore, that naringin does not affect the pharmacokinetics of dihydropyridine derivatives. Similarly, pharmacokinetics of dihydropyridine calcium antagonists was not changed by the high content of naringin in TJ-35.

In summary, TJ-35 did not affect pharmacokinetics of nicardipine. It is concluded, therefore, that the coadministration of nicardipine and TJ-35 does not increase the bioavailability of nicardipine and hence is not clinically efficacious. Further pharmacokinetic studies of drug interaction are going to be carried out in these laboratories.

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