Apocynum venetum Extract Does Not Induce CYP3A and P-Glycoprotein in Rats

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We investigated the effect of *Apocynum venetum* L. extract (AV) on the activity of cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp). The plasma concentration of nifedipine (NF), which is a substrate for CYP3A, did not change after oral administration with AV (3.3 mg/kg). Also, AV (3.3 and 33 mg/kg) did not affect the intestinal absorption of NF. In the rats treated with multiple administrations (15 mg/kg/d) of St. John's wort extract (SJW) for 2 weeks, the plasma concentration of NF after oral administration was significantly decreased. On the other hand, there was no significant differences in the pharmacokinetic parameters of NF between AV-treated (3.3 mg/kg/d) and none-treated rats. Furthermore, the intestinal absorption of methylprednisolone, which is a substrate for P-gp, was not affected by AV treatment for 2 weeks. These results suggest that, unlike SJW, the recommended dose of AV (3.3 mg/kg/d) would not influence hepatic CYP3A and intestinal P-gp in rats.

Key words *Apocynum venetum*; St John's wort; CYP3A; P-glycoprotein; rat

Apocynum venetum L. (Luobuma in Chinese, Apocynaceae) is a wild shrub grown in Asia, and its leaves have been used as a tea in China, Japan and other Asian countries. The leaves of *Apocynum venetum* L. have been used in traditional Chinese medicine. It has been reported that the extract from the leaves of *Apocynum venetum* L. (AV) exerts various pharmacological effects including diuretic,¹⁾ antihypertensive and antihyperlipidemic,²⁾ and sedative effects.³⁾ Recently, it has been shown that AV has an antidepressant effect comparable to that of imipramine, a tricyclic antidepressant, in rats.⁴⁾

The extract of St John's wort (SJW) is a commonly used herbal medicine for antidepressant⁵⁾ in Europe and U.S.A. It is considered that hyperoside in SJW would have antidepressant effects.^{6,7)} On the other hand, the long-term intake of SJW is known to induce hepatic cytochrome P450 (CYP) 3A4 and the intestinal P-glycoprotein (P-gp) in humans,⁸⁾ and causes a decrease in the blood concentrations of cyclosporin,⁹⁾ indinavir¹⁰⁾ and digoxin.¹¹⁾ However, it has not yet been revealed what compound(s) in SJW is capable of affecting metabolic enzymes and transporters. AV contains a number of phenolic compounds,¹²⁾ and hyperoside and isoquercitrin⁴⁾ are also contained in SJW. In recently, AV has come onto the market as a health food in Japan and U.S.A. Thus it is considerable that this herbal extracts would be used for an antidepressant substitute for SJW. However, the drug interaction with *Apocynum* has not been revealed even in animal model so far. In this study, we investigated the effect of AV on the drug disposition of the substrate drugs for CYP3A and/or P-gp in rats.

MATERIALS AND METHODS

Chemicals AV and SJW were supplied by Tokiwa Phytochemical Co. (Chiba, Japan). Nifedipine (NF) and methylprednisolone (MP) were obtained from Wako Pure Chem. Ind. (Osaka, Japan) and Sigma Chem. Co. (St. Louis, MO,

U.S.A.), respectively. All other reagents were of the highest grade available.

Animals Male wistar rats (Hokudo, Sapporo, Japan) were used in all studies. In this study, principles of good laboratory animal care were followed and animal experimentation was performed in compliance with Guidelines for the Care and Use of Laboratory Animals in Health Sciences University of Hokkaido, accredited by the "Principles of Laboratory Animal Care" (NIH publication #85-25, revised 1985).

Oral Administration of Nifedipine Rats aged 8 to 9 weeks and weighing 250 to 300 g were fasted 15–18 h before the experiment with free access to water. They were then anesthetized by pentobarbitone (40 mg/kg) and administered NF (0.4 mg/kg, about 0.5 ml) dissolved in Dulbecco's phosphate buffer solution (D's PBS; 137 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄ and 5 mM D-glucose, pH 6.5) with or without AV (3.3 mg/kg) by gastric intubation. After administration of drug, 0.5 ml of blood was collected from the femoral vein with a heparinized syringe at 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. The blood was immediately centrifuged at 1500×g for 10 min to obtain plasma samples.

To clarify the effect of multiple dosing of AV and SJW, rats aged 6 weeks were orally administered AV (3.3 mg/kg) or SJW (15 mg/kg) suspended in distilled water once a day at 10:00 am for 2 weeks. The dose of each herbal extract was decided according to the recommended dose for humans (AV: 200 mg/d, SJW: 900 mg/d). After continuous administration, the rats were fasted for 15–18 h and administered NF (0.4 mg/kg) without herbal extract under anesthesia. The blood was also collected in the same manner as that stated above.

In Situ Loop Experiments Absorption experiments were carried out using an *in situ* loop technique as described previously¹³⁾ with a minor modification. Fasted rats aged 8 to 10 weeks were anesthetized by pentobarbital and a jejunal loop (about 20 cm from the pylorus, 10 cm in length) was

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prepared. The bile duct was tightly ligated in all experiments. After gently washing out the contents of the loop with saline, 1 ml of D's PBS containing NF (0.4 mg/kg) with or without the herbal extract was injected into the loop. The dose of AV was 3.3 and 33 mg/kg, and SJW was 15 mg/kg. After 30 min, the loop was cut off and the contents of the loop were emptied into a 10-ml volumetric flask. The mucosal side of the loop was rinsed with a buffer to give a volume of 10 ml. Blood (0.5 ml) was collected at 15 and 30 min after the experiment was started.

Everted Sac Studies The medium for all experiments was Tyrode's buffer (137 mM NaCl, 3 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 , 12 mM NaHCO_3 , 0.4 mM NaH_2PO_4 and 6 mM D-glucose) saturated with O_2/CO_2 (95:5) gas mixture after preparation. The pH of the buffer was adjusted to 7.4 with 1 M HCl or NaOH. Rats aged 6 weeks were orally administered AV (3.3 mg/kg) or SJW (15 mg/kg) suspension once a day for 2 weeks. The everted sac studies were performed as described by Goerg *et al.*¹⁴⁾ with minor modifications. The jejunum was excised under anesthesia and rinsed in ice-cold saline. The intestinal segments were slid onto a glass rod and the epithelial surface was exposed. After washing the mucosal surface with ice-cold saline, 5-cm everted jejunal segments (4 segments per rat) were isolated. These everted segments were ligated at one end. Then, 0.2 ml of fresh Tyrode's buffer was injected into the segment and the other end was ligated. The sac was sunk in the buffer containing 100 μM MP at 37 °C. Under bubbling with a gas mixture, the permeation of the substrate from the mucosal to serosal surface across the intestine was measured by sampling the serosal buffer at 15 and 30 min.

Analysis All samples were kept at -30 °C until assay. The plasma NF assay was performed according to Miyazaki *et al.*¹⁵⁾ with several modifications. A 0.1 ml of plasma sample was mixed with 0.1 ml of saline or NF solution (for standard curve), 0.2 ml of butyl *p*-aminobenzoate as an internal standard (50 ng/ml in methanol) and 0.7 ml of acetonitrile, and then vigorously mixed for 10 s. The mixture was centrifuged at 1500 $\times g$ for 10 min. An aliquot of the resulting supernatant was collected into a glass tube containing 1 ml of distilled water, and 5 ml of chloroform/acetone mixture (1:1, v/v) was added. This mixture was vigorously shaken for 10 min and centrifuged at 1000 $\times g$ for 10 min, and the organic layer was transferred to a brown glass tube. The organic solution was dried in a rotary evaporator at 40 °C. The residue was dissolved in 0.1 ml of the mobile phase, and 20 μl of the solution was injected into the HPLC system.

The contents of the jejunal loop and everted sacs were added to the same volume of methanol and vigorously mixed for 10 s. The mixture was let stand for 15 min in an ice-bath and then centrifuged at 1500 $\times g$ for 15 min. An aliquot of the resulting supernatant was applied to HPLC.

The HPLC systems for the determination of NF and MP¹³⁾ were as follows: apparatus, Shimadzu LC-10AS (Kyoto, Japan) equipped with Shimadzu 10A UV spectrophotometric detector; column, Cosmosil 5C₁₈-ARII (4.6 i.d. $\times$ 150 mm, Nacalai Tesque, Kyoto, Japan) and column temperature, 55 °C (NF) or 50 °C (MP). The mobile phase for NF was 45% methanol in 10 mM Na_2HPO_4 (pH 6.0 adjusted with HCl) and flow rate was 1.2 ml/min. For MP, the mobile phase was 40% acetonitrile in 50 mM KH_2PO_4 and flow rate was

0.8 ml/min. The wave length for NF and MP was 280 and 246 nm, respectively. The lower limits of NF and MP quantification in sample were 5 ng/ml and 20 pmol/ml, respectively. The interassay coefficients of variations of NF and MP determination were lower than 5%.

Pharmacokinetic parameters such as the maximum concentration of NF in plasma (C_{max}) and the time at C_{max} (T_{max}) were taken from the observed data. Area under the plasma concentration-time curve for 0 to 4 h ($\text{AUC}_{0-4\text{h}}$) was calculated using the linear trapezoidal rule.

All experiments were performed on 3 to 5 rats and each data represents the mean $\pm$ S.E.M. Statistical analysis was done using unpaired Student's *t*-test and $p < 0.05$ was considered to be significant. $p < 0.1$ is shown for reference.

RESULTS

Effect of Co-administration of Herbal Extract on Drug Disposition of Nifedipine To clarify the direct effects of AV to the absorption and metabolism of the substrate for CYP3A, we examined the oral administration of NF with AV in the first study. The plasma concentration profiles of NF after oral administration are shown in Fig. 1. The absorption of NF was rapid and T_{max} was 20 to 30 min with or without AV. There were no statistical differences in the pharmacokinetic parameters of NF with or without AV (data not shown).

Fig. 1. Time Courses of the Plasma Concentration of Nifedipine (0.4 mg/kg) after Oral Administration with or without *Apocynum venetum* L. Extract (3.3 mg/kg)

Each point represents the mean with S.E.M. of 3 experiments.

Fig. 2. Plasma Concentration of Nifedipine (0.4 mg/kg) during *in Situ* Loop Experiments with or without Herbal Extracts

Each column represents the mean with S.E.M. of 3 to 4 experiments.

Fig. 3. Effect of a 2-Week Treatment with St John's Wort (Left) and *Apocynum venetum* L. Extract (Right) on the Time Courses of Plasma Concentration of Nifedipine (0.4 mg/kg) after Oral Administration

Each point represents the mean with S.E.M. of 4 to 5 experiments. * Significantly different from no treatment, $p < 0.05$, $^{\dagger} p < 0.1$.

Table 1. Effect of a 2-Week Treatment with Herbal Extract on the Pharmacokinetic Parameters of Nifedipine

Treatment	$C_{\max}$ (ng/ml)	$T_{\max}$ (h)	AUC_{0-4h} (ng·h/ml)
No treatment	265.3 ± 26.1	0.33 ± 0.05	426.4 ± 54.0
AV (3.3 mg/kg/d)	216.0 ± 31.9	0.70 ± 0.36	425.5 ± 93.3
SJW (15 mg/kg/d)	$159.2 \pm 27.1^*$	0.48 ± 0.10	$254.7 \pm 56.5^{\dagger}$

Each data represents the mean $\pm$ S.E.M. of 3 to 5 rats. * Significantly different from no treatment, $p < 0.05$, $^{\dagger} p < 0.1$.

The effect of AV and SJW on the intestinal absorption of NF from rat jejunal loops was also examined. NF almost completely disappeared from loop with or without the herbal extract after 30 min. The percentage of disappearance was 98.3 ± 1.7 , 98.1 ± 2.3 , 98.9 ± 0.8 and 97.4 ± 1.5 for without herbal extract, with AV (3.3 mg/kg), AV (33 mg/kg) and SJW (15 mg/kg), respectively. Moreover, there were no significant differences in NF plasma concentration at 15 and 30 min among the four experiments (Fig. 2).

Effect of 2-Weeks Treatment with Herbal Extract on Drug Disposition of Nifedipine In the rats treated with SJW for 2 weeks, the plasma levels of NF at 45 and 60 min after oral administration were significantly lower than those of the control rats (Fig. 3, left). Furthermore, the pharmacokinetic parameters such as $C_{\max}$ and AUC_{0-4h} were also lower than for the control group (Table 1). On the other hand, only the NF plasma level at 20 min was lower in AV-treated rats (Fig. 3, right), but there were no significant differences at the other time points and in the pharmacokinetic parameters from those in the control rats.

Effect of 2-Weeks Treatment with Herbal Extract on Absorption of Methylprednisolone To reveal the effect of AV on intestinal P-gp, we examined the transport of MP as a substrate for P-gp across the everted intestine from rats treated with AV or SJW for 2 weeks. As shown in Fig. 4, there were no differences in permeation at 15 min between the control and herbal extract-treated rats. On the other hand, the permeation of MP at 30 min in SJW-treated rats, unlike AV-treated rats, was significantly greater than that in the control rats.

Fig. 4. Effect of a 2-Week Treatment with *Apocynum venetum* L. and St John's Wort Extract on the Permeation of Methylprednisolone ($100 \mu\text{M}$) across the Rat Jejunal Intestine

Each column represents the mean with S.E.M. of 3 to 4 rats. * Significantly different from no treatment, $p < 0.05$.

DISCUSSION

SJW is a commonly used herbal extract in Europe and U.S.A. The long-term intake of SJW causes to decrease in the efficacy of many drugs⁹⁻¹¹ since it induces CYP3A4 and P-gp.⁸ AV contains a lot of flavonoids such as hyperoside and isoquercitrin,⁴ which are also contained in SJW. However, there was no information as to AV-drug interaction in not only human but in animal model. It is well known that NF is metabolized by CYP3A, and the bioavailability of NF was significantly reduced by multiple administration of rifampicin¹⁶ and grapefruit juice¹⁷ since they induced the CYP3A in human and rats. Moreover, we have reported that MP was a good substrate for P-gp in Caco-2 cell.¹⁸ In the present study, therefore, we evaluated the effect of AV on the drug disposition of NF and MP, a substrate for CYP3A and P-gp, respectively, in rats. A single administration of AV did not alter the plasma concentration of NF (Fig. 1). We also examined the effect of AV and SJW on the absorption of NF using an *in situ* loop technique. The absorption of NF was not changed by the recommended or a 10-fold higher dosage of AV and SJW (Fig. 2). These results suggest that a single administration of AV at the recommended dosage does not

directly inhibit the activity of hepatic and intestinal CYP3A in rats.

Dürr *et al.* reported that intestinal P-gp and hepatic CYP3A2 were induced by the multiple administration of SJW for 2 weeks in rats.⁸⁾ Thus, we repeated the oral administration of AV and SJW for 2 weeks and then evaluated their effect on the disposition of CYP3A and P-gp substrates. After the multiple administration of SJW, the plasma concentrations of NF became significantly lower than those in the control rats (Fig. 3), which is consistent with the previous report that SJW induced hepatic CYP3A4 in humans. On the other hand, there were no differences in the plasma concentration of NF between the AV-treated and control rats. These results strongly suggest that, unlike SJW, the recommended dosage of AV does not induce CYP3A in rats. In our previous report, we found that the intestinal absorption of corticosteroid analogues was restricted by intestinal P-gp.¹⁰⁾ Thus we evaluated the effect of a long-term treatment of AV on the activity of intestinal P-gp using MP. The permeation of MP from the mucosal to serosal side of the rat intestine did not differ between the AV-treated and control rats (Fig. 4) in spite of an observation that MP transport was greatly changed in SJW-treated rats. It is still unclear what compound(s) is the cause of CYP3A and P-gp induction by SJW, however a recommended dosage of AV did not change the disposition of the substrate drugs for CYP3A and P-gp in rats. Leaves of *Apocynum* have been traditionally used as a tea, however, there had been no report regarding the drug interactions of this herb. The present results suggest that AV lacks the ability to modify the activity of CYP3A and P-gp of rats *in vivo*. More detailed investigations about the effects of AV to the other CYP and transporters in rats and human should be reported in the future.

In conclusion, our present study shows for the first time that neither a single or multiple administration of the recom-

mended dose of AV (3.3 mg/kg/d) changes the drug disposition *via* CYP3A and P-gp in rats.

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