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The in-vivo effect of bakumondo-to (TJ-29), a traditional Japanese medicine used for treatment of chronic airway disease, on cytochrome P450 1A2, xanthine oxidase and *N*-acetyltransferase 2 activity in man

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Abstract

In Japan, patients with chronic airway disease are administered bakumondo-to (TJ-29), a mixture of six herbal components. We have assessed the effects of TJ-29 on the activities of cytochrome P450 (CYP) 1A2, xanthine oxidase and *N*-acetyltransferase 2 in 26 healthy subjects under a double-blind, randomized, placebo-controlled cross-over study design. The baseline activities of the three enzymes were assessed by the respective urinary metabolic ratios of an 8-h urine sample after an oral 150-mg dose of caffeine. Thereafter, the subjects received a thrice-daily 3.0-g dose of TJ-29 or placebo for seven days, and underwent the same caffeine test on the post-dose days 1 and 7. No statistically significant difference was observed in the activity of the three enzymes between those at baseline, and on day 1 after dosing with TJ-29 or placebo. The mean activity of CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 tended to be lower on day 7 after dosing with TJ-29 compared with those at baseline and on day 7 after dosing with placebo. However, these changes were not statistically significant in CYP1A2 ($P = 0.120$), xanthine oxidase ($P = 0.123$) or *N*-acetyltransferase 2 ($P = 0.056$). In conclusion, TJ-29 did not appear to substantially affect the activity of CYP1A2, xanthine oxidase or *N*-acetyltransferase 2 in man.

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Introduction

Herbal medicines are increasingly used in the world, and herb–drug interactions are recognized as a clinically important problem (Fugh-Berman 2000; Izzo & Ernst 2001; Ioannides 2002; Zhou et al 2003). In Japan, 148 kinds of traditional Chinese herbal medicines (Kampo medicines) have been approved by the government and are listed on the 'National Health Insurance Drug Tariff', whereas little information about herb–drug interaction is available.

Bakumondo-to (TJ-29, Chinese name: Mai-men-dong-tang) is a popular Kampo medicine, which is widely used in Japan to treat chronic airway diseases, such as bronchitis and asthma (Tamaoki et al 1993; Miyata et al 1998; Aizawa et al 2003). TJ-29 can be administered for a couple of days or for years, depending on the indicated disease and the symptoms. It is often co-administered with other bronchodilators such as theophylline, which is metabolized by cytochrome P450 (CYP) 1A2 with a narrow therapeutic range (Tjia et al 1996; Weinberger & Hendeles 1996; Rendic & Di Carlo 1997; Barnes 2003). The caffeine test is used widely for assessing the CYP1A2 activity in the herb–drug interaction screening survey, because of its safe and non-invasive properties (Kalow & Tang 1993; Saruwatari et al 2003; Wenk et al 2004). For this reason, we have used a caffeine test for assessing the effect of TJ-29 on CYP1A2 activity.

Xanthine oxidase has been implicated as the generator of active oxygen and as a potential target in respiratory diseases such as chronic obstructive pulmonary disease (COPD) (Ichinose et al 2003). TJ-29 has been used for the treatment of COPD accompanying viscous sputum. However, whether TJ-29 would affect the xanthine oxidase activity has remained totally unknown. By adopting the caffeine test, the

change in the activity of *N*-acetyltransferase 2 could be simultaneously evaluated with the activity of CYP1A2 and xanthine oxidase using the same urine sample (Kalow & Tang 1993).

In this report, we have demonstrated the effect of TJ-29 on the activities of CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 in healthy volunteers under a double-blind, randomized, placebo-controlled cross-over design.

Materials and Methods

Chemicals

TJ-29 was obtained from Tsumura Co. (Tsumura Bakumondo-to Extracts, Tokyo, Japan). A 9-g sample of TJ-29 contained 6.0 g dried extract, which was prepared from the boiled water extracts of six herbal components as follows: 10.0 g Ophiopogonis tuber (*Ophiopogon japonicus* Ker-Gawler), 5.0 g brown rice (*Oryza satiba* L.), 5.0 g Pinelliae tuber (*Pinellia ternata* Breitenbach), 3.0 g Jujube fruit (*Ziziphus jujuba* Miller), 2.0 g Ginseng root (*Panax ginseng* C. A. Meyer) and 2.0 g Glycyrrhiza root (*Glycyrrhiza uralensis* Fischer) (TSUMURA Bakumondo-to Extract Granules for Ethical Use). 1,7-Dimethyl-uric-acid (17U), 1-methylxanthine (1X) and 1-methyl-uric-acid (1U) were purchased from Sigma Chemical Co. (St Louis, MO).

5-Acethyl-amino-6-formylamino-3-methyluracil (AFMU), a precursor of 5-acethylamino-6-amino-3-methyluracil (AAMU), was purchased from Welfide Co. (Osaka, Japan).

Placebo

A mixture of 45 g lactose (Yoshida Pharmaceutical, Tokyo, Japan), 35 g potato starch (Yoshida Pharmaceutical, Tokyo, Japan), 10 g hydroxypropylcellulose-L (Nippon Soda, Tokyo, Japan) and 5 g hydroxypropyl-starch (Freund Industrial, Saitama, Japan) passed through a sieve of DIN-Nr.8 was obtained by mixing in a mortar and pestle. After 22 mL 0.55% caramel (Amano Jitsugyo, Hiroshima, Japan) aqueous solution was added, the mixture was blended to a uniform colour in a mortar and pestle. The mixture was then granulated using a granulating machine. After drying, the granules, which were passed through a sieve of DIN-Nr.5 and remained on another sieve of DIN-Nr.8, were provided as a placebo.

Subjects

The Institutional Review Board of Kumamoto University School of Pharmacy approved the study. All of the volunteers (all university students) provided written informed consent to participate in the study.

We studied 26 healthy Japanese volunteers (10 females and 16 males), ranging in age from 21 to 25 years (median, 22 years); the weight range of the subjects was 40–83 kg (mean $\pm$ s.d., 56.3 ± 11.4 kg). All volunteers were judged to be healthy according to their medical history and physical examination, blood chemistry and urinalysis. Each volunteer was required to be a non-smoker. Participants were excluded for the following reasons: allergy to TJ-29, any herb, any non-herbal medicine or caffeine; history of any infectious disease within four weeks before enrollment; use of TJ-29 or other herbal medicines within four weeks; use of prescription or over-the-counter medications or alcohol within two days; use of an investigational drug within two months; history of alcohol or drug abuse; and current pregnancy or its suspicion. Grapefruit or grapefruit juice intake was prohibited at least one week before and throughout the study.

Study design

This was a double-blind, randomized, placebo-controlled, two-period cross-over study with a two-week wash-out period between the two trial phases. The 13 subjects were randomly allocated to either of the two dosing sequences (placebo–TJ-29 or TJ-29–placebo). Each subject received placebo or TJ-29 for one week, and then took the other treatment for one week after the two-week wash-out period.

From 48 h before the first caffeine test through to the end of the study, volunteers were not allowed to eat any food or beverage containing xanthines (e.g. coffee, tea, Japanese tea, cola, chocolate). Subjects were instructed to take a thrice-daily 3.0-g dose (a standard dose used for treatment of chronic airway disease) of TJ-29 or placebo before a meal for seven days. The caffeine test was performed three times: two days before (baseline), and on the first day (day 1) and seventh day (day 7) after the start of TJ-29 or placebo dosing. Compliance was assessed by self-reporting of missed doses at the end of the study.

Blinding

The randomization code was kept locked by the study director, unless a serious adverse event occurred and identification of the study medication was required.

Caffeine test

After emptying their bladders, the subjects received an oral dose of 150-mg caffeine before they went to sleep. An overnight urine sample was collected from each subject (approximately from 2300 h to 0700 h). The mean ($\pm$ s.d.) length of time for urine collection was 8.4 ± 1.0 h. The urine sample (1 mL) was acidified using ascorbic acid (10 mg mL^{-1}) and was stored at $-20^{\circ}C$ until the HPLC analyses of the caffeine metabolites. For the data correction, we applied spot urine samples before taking caffeine on day 1 and day 7 after dosing with TJ-29 or placebo in the 26 subjects.

Analytical procedure

Urinary concentrations of three caffeine metabolites, 17U, 1U, and 1X, were quantified by the validated HPLC

analysis as reported by Saruwatari et al (2003). After complete conversion of AFMU into AAMU at pH 10, the AAMU was measured by an HPLC with an ultraviolet detection as described previously (Saruwatari et al 2003).

Statistical methods

According to the criteria of the caffeine test previously established (Kalow & Tang 1993; Streetman 2000), the activity of CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 was assessed by use of the molar concentration ratios (AAMU + 1U + 1X)/17U, 1U/(1U + 1X) and AAMU/(AAMU + 1X + 1U), respectively. The one-way or two-way repeated measure analysis of variance was used to analyse all of the data. A P value < 0.05 was considered to be statistically significant. The power analysis was used to estimate the ability to detect significant distances between the smallest and largest of the mean values. These statistical analyses were performed with an SPSS (version 11.0J, SPSS Inc., Chicago, IL). The sample size calculation was used by the method of Cohen (1969).

Results

All enrolled subjects completed the study protocol and reported that they had taken all dispensed study medications as directed. TJ-29 was well tolerated without any adverse reactions. The randomization code was opened after all analytical data had been collected.

The mean (± s.d.) and individual values of the three drug-metabolizing enzyme activities at baseline, and on day 1 and day 7 after dosing with TJ-29 are shown in Figure 1. There were no significant differences among the three phases in the mean activities of CYP1A2 ($P = 0.368$), xanthine oxidase ($P = 0.127$) and *N*-acetyltransferase 2 ($P = 0.127$), respectively.

Figure 2 shows the mean (± s.d.) and individual values of each enzyme activity at baseline, and after the first day dosing with placebo or TJ-29. No statistically significant differences were observed in the three enzyme activities between those at baseline, and on day 1 after dosing with TJ-29 or placebo in the mean activities of CYP1A2 ($P = 0.995$), xanthine oxidase ($P = 0.914$) and *N*-acetyltransferase 2 ($P = 0.073$), respectively.

The mean (± s.d.) and individual values of each enzyme activity at baseline, and after the 7-day dosing with placebo or TJ-29 are shown in Figure 3. Although the mean activity of CYP1A2 obtained from the TJ-29-treated period decreased by 14.9% from the baseline and 18.8% from the placebo-treated period, these changes did not reach a statistically significant difference ($P = 0.120$). The mean activity of xanthine oxidase also decreased in the TJ-29-treated period compared with that of the baseline or placebo-treated period, with the mean reduction values of 15.4% and 4.3% compared with those from the baseline and

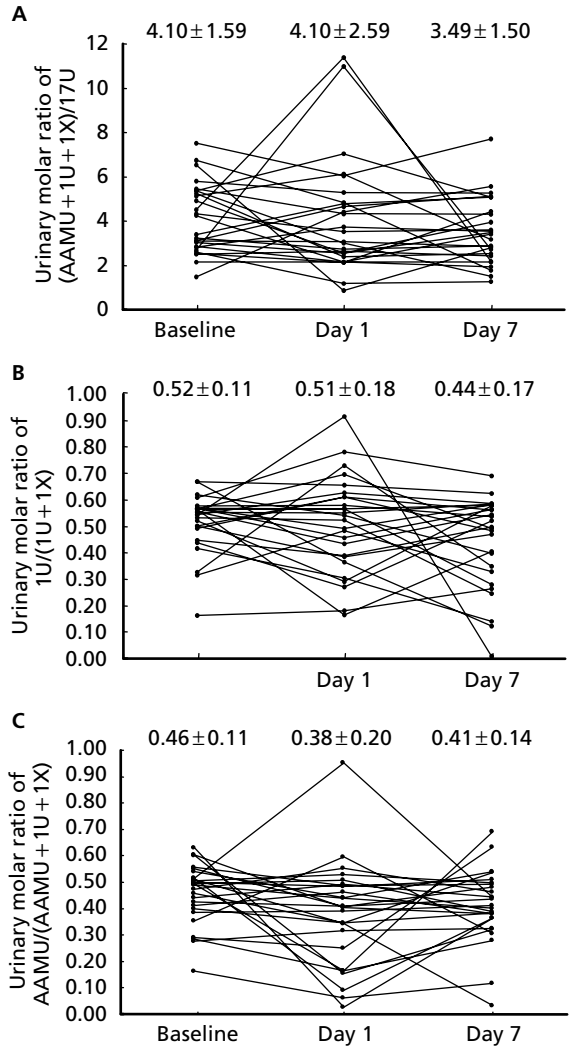

Figure 1 Individual values of the urinary molar concentration ratios of (AAMU + 1U + 1X)/17U for CYP1A2 (A), 1U/(1U + 1X) for xanthine oxidase (B) and AAMU/(AAMU + 1U + 1X) for *N*-acetyltransferase 2 (C) activities in 26 healthy subjects before (baseline), and on the first day (day 1) and on the seventh day (day 7) after dosing with TJ-29. Mean enzyme activities are expressed as mean ± s.d. in the upper part of the figure. AAMU, 5-acethyl-amino-6-amino-3-methyluracil; 1U, 1-methyl-uric-acid; 1X, 1-methyl-xanthine; 17U, 1,7-dimethyl-uric-acid; CYP1A2, cytochrome P450.

the placebo-treated period, respectively. However, these reductions were also not statistically significant ($P = 0.132$). Although the mean activity of *N*-acetyltransferase 2 tended to decrease on day 7 after dosing with TJ-29 by 14.6% from the placebo-treated period, this change did not reach statistical significance ($P = 0.056$).

When the data on the mean metabolic ratios for CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 were compared between the 16 male and 10 female subjects, gender-related differences were not statistically detected ($P > 0.10$, data not shown).

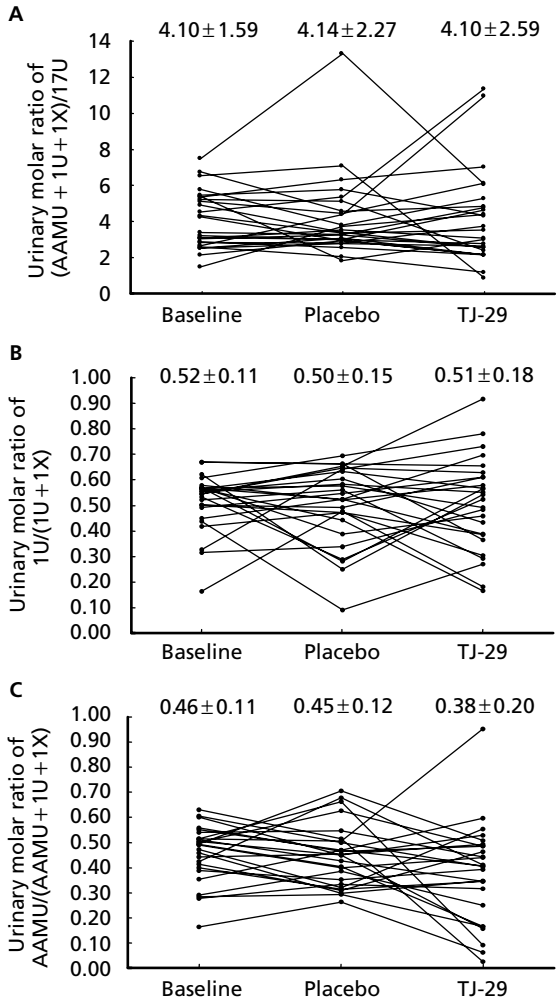

Figure 2 Individual values of the urinary molar concentration ratios of (AAMU + 1U + 1X)/17U for CYP1A2 (A), 1U/(1U + 1X) for xanthine oxidase (B) and AAMU/(AAMU + 1U + 1X) for *N*-acetyltransferase 2 (C) activities in 26 healthy subjects before (baseline), and on the first day (day 1) after dosing with placebo and TJ-29. Mean enzyme activities are expressed as mean ± s.d. in the upper part of the figure. For the abbreviations, see the legend of Figure 1.

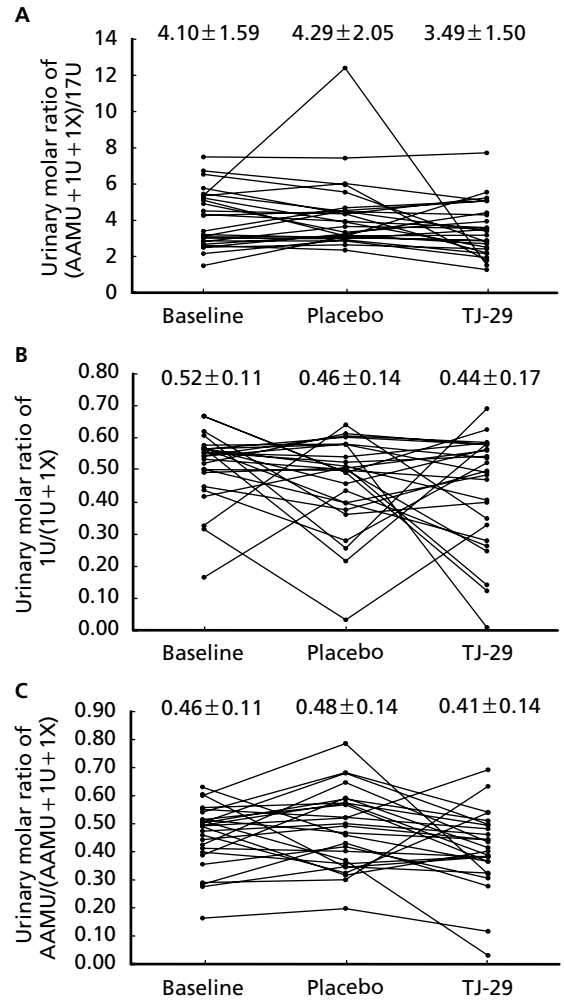

Figure 3 Individual values of the urinary molar concentration ratios of (AAMU + 1U + 1X)/17U for CYP1A2 (A), 1U/(1U + 1X) for xanthine oxidase (B) and AAMU/(AAMU + 1U + 1X) for *N*-acetyltransferase 2 (C) activities in 26 healthy subjects before (baseline), and on the seventh day (day 7) after dosing with placebo and TJ-29. Mean enzyme activities are expressed as mean ± s.d. in the upper part of the figure. For the abbreviations, see the legend of Figure 1.

Discussion

As a safe and non-invasive test, urinary caffeine metabolic ratios have been widely used for assessing the CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 activities in herb–drug interaction screening surveys (Saruwatari et al 2003; Wenk et al 2004). Although several equations have been proposed for assessing the CYP1A2 activity, the (AAMU + 1U + 1X)/17U ratio in an 8-h urine sample (used in this study) has been considered as the best CYP1A2 index among the proposed urinary metabolic ratios (Kalow & Tang 1993; Streetman et al 2000).

The 7-day repeated dosing with TJ-29 to healthy volunteers showed no significant effect on the activity of CYP1A2, xanthine oxidase or *N*-acetyltransferase 2 assessed by the caffeine test. We had demonstrated previously an inhibitory effect of sho-saiko-to on the CYP1A2 and xanthine oxidase activities in healthy volunteers by using the same caffeine test (Saruwatari et al 2003). The difference in the pharmacologically active ingredients of TJ-29 and sho-saiko-to is shown in Table 1. Three flavonoids (i.e. baicalein, wogonin and oroxylin A) of Scutellaria root have been reported to inhibit the CYP1A2 activity in human liver microsomes (Kim et al 2002) (Table 1). In addition, baicalein inhibits the xanthine oxidase activity in-vitro (Shieh et al 2000) (Table 1). TJ-29 does not contain Scutellaria root, and so these flavonoids of Scutellaria root may be the main active ingredients that inhibit the CYP1A2 or xanthine oxidase activity by sho-saiko-to. Nevertheless, whether

Table 1 Herbal compositions and major active ingredients of TJ-29 (bakumondo-to) and sho-saiko-to, and their in-vitro effects on CYP1A2 and xanthine oxidase activity reported in the literature

Herbal components	Species names	Composition (%; w/w)		Main active ingredients	Effects of ingredients on two enzyme activities* (species)	References
		TJ-29	Sho-saiko-to			
Ophiopogonis tuber	*Ophiopogon japonicus* Ker-Gawler	37.1		Ophiopogonins A–D, B′–D′ Methylophiopogonanones A, B		
Brown rice	*Oryza satiba* L.	18.5		Starch		
Pinellia tuber	*Pinellia ternata* Breitenbach	18.5	20.8	Adenine		
Jujube fruit	*Ziziphus jujuba* Miller	11.1	12.5	Cyclic AMP		
Ginseng root	*Panax ginseng* C. A. Meyer	7.4	12.5	Unknown ingredients Ginsenoside Rd Ginsenoside Rb1 Ginsenoside Rg1	CYP1A2 ↓ (human)	Chang et al (2002)
Glycyrrhiza root	*Glycyrrhiza uralensis* Fischer	7.4	8.3	Glycyrrhizin Liquiritigenin Isoliquiritigenin Liquiritin Glycycoumarin Glycyrrhetic acid	XO ↓ (bovine) XO ↓ (bovine)	Kong et al (2000) Kong et al (2000)
Bupleurum root	*Bupleurum falcatum* Linne		29.2	Saikosaponin-d		
Scutellaria root	*Scutellaria baicalensis* Georgi		12.5	Baicalin Baicalein Wogonin Oroxylin A	CYP1A2 ↓ (human) XO ↓ (unknown) CYP1A2 ↓ (human) CYP1A2 ↓ (human)	Kim et al (2002) Shieh et al (2000) Kim et al (2002) Kim et al (2002)
Ginger rhizome	*Zingiber officinale* Roscoe		4.2	6-Gingerol 6-Shogaol Zingerone		

↓, Inhibition; CYP, cytochrome P450; XO, xanthine oxidase. *There was no report on the in-vitro effect of active ingredients on the *N*-acetyltransferase 2 activity.

the inhibitory effects of sho-saiko-to would come only from those flavonoids remains obscure from our study, because the possibility is not totally negated that other ingredients contained in these two herbal medicines might inhibit the enzymatic activity.

CYP1A2 is one of the important enzymes in the metabolism of several drugs whose content in human liver microsomes has been reported to be approximately 10%. It metabolizes theophylline, which shows a narrow therapeutic index (5–20 $\mu g\,mL^{-1}$) (Tjia et al 1996; Weinberger & Hendeles 1996; Rendic & Di Carlo 1997; Barnes 2003). TJ-29 has been used widely in Japan (annual sales in 2002: approximately 21 million US$). TJ-29 exhibits anti-inflammatory and anti-tussive effects on small airways (Tamaoki et al 1993; Miyata et al 1998; Aizawa et al 2003), and is frequently co-prescribed with theophylline in patients with asthma or COPD accompanied by cough (TSUMURA bakumondo-to Extract Granules for Ethical Use). To our knowledge, no adverse event has been reported in patients using theophylline with TJ-29. Although our results were obtained from healthy volunteers, we are tempted to assume that the combination therapy of theophylline and TJ-29 would be clinically safe in terms of the interaction potential in patients with asthma or COPD.

Xanthine oxidase has been reported to produce active oxygen in respiratory diseases such as COPD (Ichinose et al 2003). No significant change was observed in the xanthine oxidase activity after the 7-day repeated dosing of TJ-29 in this study. However, whether any inhibitory effect of TJ-29 on the xanthine oxidase activity might be involved in the clinical effectiveness of COPD remains totally obscure from this study, because we did not measure the topical xanthine oxidase activity, such as that in bronchoalveolar lavage fluid.

N-Acetyltransferase 2 is known to be responsible for the genetically determined acetylation metabolism of commonly used drugs (e.g. isoniazid, sulfamethazine, procainamide) (Evans 1989). Our results showed that the mean activity of *N*-acetyltransferase 2 tended to decrease after the 7-day repeated dosing with TJ-29 ($P = 0.056$). However, to our knowledge, there is no report concerning the in-vivo interaction between TJ-29 and *N*-acetyltransferase 2 substrates in man. Therefore, we assumed that the mean reduction value of up to 15% in the *N*-acetyltransferase 2 activity by TJ-29 might not be clinically significant, although a study with a larger number of subjects would definitely be required.

For the differences among the mean enzymatic activities at the baseline and the 7-day dosing with placebo and TJ-29, the statistical powers (1-β) were estimated to be 43% for CYP1A2, 41% for xanthine oxidase and 57% for *N*-acetyltransferase 2. According to the method of Cohen (1969), the sample size calculation showed that 58, 28 and 37 subjects would be needed to be statistically significant ($\alpha = 0.05$ and $\beta = 0.80$) for the differences in the CYP1A2, xanthine oxidase and *N*-acetyltransferase 2 activities, respectively. This suggested the possibility that 43 to 59% probability at the type II error (with an α-level of 0.05) could not be negated, as well as that our sample size (i.e. $n = 26$) was limited, particularly for CYP1A2 and *N*-acetyltransferase 2. Therefore, to avoid type II error, a larger sample size would be required to elucidate the lack of significant difference obtained from our study. Nevertheless, with the study limitations as mentioned above, we anticipate that TJ-29, in contrast to sho-saiko-to (Saruwatari et al 2003), would not substantially affect the CYP1A2 and xanthine oxidase activities.

Conclusion

We have demonstrated that in volunteers, pre-treatment with TJ-29 for one or seven days did not appear to substantially affect the activity of CYP1A2, xanthine oxidase or *N*-acetyltransferase 2. Our findings might be useful to judge the possible interaction potential of TJ-29 with the drugs metabolized by these three enzymes, particularly by CYP1A2. Further clinical studies in man are necessary to elucidate any possible interactions and effects that TJ-29 or other Kampo medicines might have with Westernized pharmaceutical drugs in light of the increasing use of herbal medicines all over the world.

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