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Impacts of Kampo medicine on induction of CYP3A4 and ABCB1 in gastrointestinal cell model LS180

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ABSTRACT

Background & Aim: The clinical use of some Kampo medicines has increased rapidly, and opportunities to be used concomitantly with Western medicines more frequently. Although the inhibition of cytochrome P450(CYP)-mediated drug metabolism and ABCB1-mediated transport by Kampo medicine has been reported, little information is available regarding the induction of CYP enzymes or P-glycoprotein—which is encoded by the highly polymorphic ATP-binding cassette transporter B1 (*ABCB1*) gene—by Kampo medicine. This study aimed to evaluate the induction of CYP enzymes and ABCB1 using Kampo medicines.

Experimental: Four Kampo medicines, namely Saireito, Shosaikoto, Goreisan, and Daikenchuto, were selected. The induction of *CYP3A4* and *ABCB1*mRNA expressionwas evaluated in human-derived colon adenocarcinoma LS180 cells, which are an established model for investigating gene induction mediated by the pregnane X receptor.

Results: Exposure to Saireito caused a dose-dependent increase in *CYP3A4* mRNA expression. A significant increase in *CYP3A*4 mRNA expression was also observed with Goreisan and Daikenchuto, but not with Shosaikoto. Exposure to Saireito, Shosaikoto, and Goreisan significantly upregulated the expression of *ABCB1* mRNA in a dose-dependent manner, but exposure to Daikenchuto had no such effect. These results indicate the differing induction effects of Kampo medicines and the distinct profiles of *CYP3A4* and *ABCB1*, suggesting the upregulation of CYP3A4 or ABCB1 expression by Kampo medicines in enterocytes.

Recommended applications/industries: Collectively, our results show that Kampo medicines can potentially induce the expression of CYP enzymes and ABCB1, and provide useful clinical information on the safety and efficacy of the combined use of Kampo and Western medicines.

1. Introduction

Traditional Japanese Kampo medicines have been approved by the Japanese Ministry of Health, Labor, and Welfare for the treatment of various diseasesin Japan for many years. The clinical use of some Kampo medicines has increased rapidly, and they are now frequently used concomitantly with Western medicines (Ernst, 2004; Motoo *et al.*, 2011; Pal and Shukla, 2003; Terasawa, 2004). In clinical situations where Kampo

and Western medicines are combined, it is important to elucidate any potential drug-drug interactions (DDIs) to ensure the safety and efficacy of their combined use (Fugh-Berman, 2000).

Previous studies have shown that Kampo medicines and their components inhibit cytochrome P450 (CYP)-mediated drug metabolism (Ito *et al.*, 2008; Iwanaga *et al.*, 2010; Iwata *et al.*, 2004b; Iwata *et al.*, 2004a; Takiyama *et al.*, 2022) and P-glycoprotein (ABCB1)-mediated transport (Hyuga *et al.*, 2012; Matsumoto *et al.*, 2018; Satoh *et al.*, 2009; Takara *et al.*, 2005). Consequently, clinical DDIs may occur. However, serious DDIs between Kampo and Western medicines do not occur in clinical practice. The reason for this remains unclear.

The herbal extract of St. John's wort is known to induce CYP enzymes or ABCB1, which can lead to herb-drug interactions (Nicolussi *et al.*, 2020). However, little information is available regarding the induction of CYP or ABCB1 by Kampo medicines. Therefore, the inhibitory effects of Kampo medicine on CYP enzymes or ABCB1 may be masked by the induction effects of Kampo medicines.

In this study, four Kampo medicines were investigated, namely Saireito (TJ-114), Shosaikoto (TJ-9), Goreisan (TJ-17), and Daikenchuto (TJ-100), which are widely used in Japan and frequently combined with Western drugs (Watanabe *et al.*, 2011). To verify whether these Kampo medicines can induce CYP3A4or ABCB1, their effects on the expression of *CYP3A4* and *ABCB1* mRNA were examined using human colon adenocarcinoma LS180 cells as an intestinal cell model.

2. Materials and Methods

2.1. Chemicals

Commercially available Kampo medicines, namely Saireito (TJ-114), Shosaikoto (TJ-9), Goreisan (TJ-17), and Daikenchuto (TJ-100), were purchased from Tsumura & Co. (Tokyo, Japan). All other reagents were obtained commercially and were of analytical grade, requiring no further purification.

2.2. Preparation of Kampo test solutions

Commercially available Shosaikoto, Goreisan, and Daikenchuto containing 2.5 g of dried Kampo extract per package, as well as Saireito containing 3.0 g of dried Kampo extract per package, were used in this study (Table 1, TSUMURA & CO, Tokyo, Japan). Dried Kampo extract (400 mg) was suspended in 10 mL of distilled water and methanol (1:1). Each suspension was sonicated and vortexed three times for 10 minutes. Each suspension was centrifuged at 9000×*g* for 5 minutes at room temperature and the supernatant was 10-fold diluted with methanol. Aliquots of the diluted supernatants were divided into microtubes and distilled in vacuo using a rotary evaporator. The resultingextract was re-dissolved in a complete culture medium, and the test solution was adjusted to the amount corresponding to 0.4, 4, and 8 mg of dried Kampo extract.

Table 1. Contents and ingredients of Kampo medicines used.

Kampo medicine	Ingredient	Content (ratio)
Saireito	Bupleuri radix	7.0
	Alismatis rhizome	5.0
	Pinelliae tuber	5.0
	Scutellariae radix	3.0
	Atractylodis lanceae rhizome	3.0
	Zizyphi fructus	3.0
	Polyporus	3.0
	Ginseng radix	3.0
	Poria	3.0
	Glycyrrhizae radix	2.0
	Cinnamomi cortex	2.0
	Zingiberis rhizome	1.0
Shosaikoto	Bupleuri radix	7.0
	Pinelliae tuber	5.0
	Scutellariae radix	3.0
	Zizyphi fructus	3.0
	Ginseng radix	3.0
	Glycyrrhizae radix	2.0
	Zingiberis rhizome	1.0
Goreisan	Alismatis rhizome	4.0
	Atractylodis lanceae rhizoma	3.0
	Polyporus	3.0
	Poria	3.0
	Cinnamomi Cortex	1.5
Daikenchuto	Zingiberis Processum Rhizoma	5.0
	Ginseng radix	3.0
	Atractylodis Lanceae Rhizoma	2.0

2.3 Cells and cell culture

Human-derived colon adenocarcinoma LS180 cells, a well-established model for investigating gene induction mediated by PXR or AhR (Brandin *et al.*, 2007; Okada *et al.*, 2017; Takara *et al.*, 2003; Weiss *et al.*, 2013),

were used in this study. LS180 cells were grown in a complete culture medium composed of α-minimal essential medium (α-MEM, Cat. No. M4655, Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich), 100 U/mL penicillin G, 100 μg/L streptomycin sulfate, and 0.1mM non-essential amino acids (Invitrogen, Waltham, MA, USA). The cells were seeded at a density of 2× 10^6 cells/25 cm^2 flask in 10 mL of complete α-MEM and subcultured every 3 or 4 days.

2.4. Quantitative reverse transcription (RT)-PCR

LS180 cellswere seeded in plastic culture dishes (60 mm in diameter) with 5 mL of complete culture medium. The culture medium was replaced every 2 days with fresh complete culture medium without any drugs. The cells were pre-cultured for 10 days in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Subsequently, the culture medium was exchanged with or without Kampo medicine (0.4, 4, or 8 mg/dish/5 mL), and further incubated for 24 h in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Total RNA was extracted from the cells using the GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich), and the mRNA expression levels of *CYP3A4* and *ABCB1* were measured using quantitative RT-PCR. Three independent sample sets were analyzed (Kitada *et al.*, 2008; Minegaki *et al.*, 2013). Total RNA (500 ng) was used for reverse transcription using a PrimeScript™ RT reagent kit (Takara Bio Inc., Shiga, Japan) and a thermal cycler (i-Cycler, Bio-Rad, Hercules, CA, USA). The reverse transcription reaction was conducted in 20 μL of reaction buffer at 37°C for 15 min and terminated by heating at 85°C for 5 s, followed by cooling at 4°C. Real-time PCR was performed using a 7500 Fast Real-Time PCR system (Applied Biosystems, Waltham, MA, USA) and SYBR Premix Ex Taq™ (Takara Bio, Shiga, Japan), with β-Actin (*ACTB*) as an internal standard. The primer sequences used were CYP3A4_F; 5'-CATTCCTCATCCCAATTCTTGAAGT-3'; CYP3A4_R; 5'-CCACTCGGTGCTTTTGTGTATCT-3'; ABCB1_F; 5'-TTCCTTCACCCAGGCAATG-3'; ABCB1_R; 5'-ATGAGTTTATGTGCCACCAAGTAG-3';ACTB_F; 5'-TCATGAAGTGTGACGTGGACATC-3'; ACTB_R; 5'- TGCATCCTGTCGGCAATG-3', and were synthesized by GeneDesign, Inc. (Ibaraki, Japan). The PCR reaction was performed at 95°C for 30 s, followed by 40 cycles at 95°C for 3 s and 60°C for 30 s, and a dissociation curve analysis was conducted. To compare the relative expression of target mRNA levels, the comparative CT method was used as previously described (Kitada *et al.*, 2008; Minegaki *et al.*, 2013).

2.5. Statistical analysis

Comparisons among the four groups were performed using non-repeated measures analysis of variance followed by Dunnett's test using JMP® Pro 15.2.0. (SAS Institute Japan Ltd., Tokyo, Japan). A *p*-values of less than 0.05 (two-tailed) was considered significant.

3. Results and discussion

3.1. Effects of Kampo medicine on the expression level of CYP3A4 mRNA

To investigate the effects of Kampo medicines on CYP enzyme and ABCB1 expression, we examined the expression level of *CYP3A4* mRNAin LS180 cells following their exposure to Saireito, Shosaikoto, Goreisan, or Daikenchuto. Exposure to Saireito caused a dose-dependent increase in the expression level of *CYP3A4* mRNA (Figure 1a). Goreisan and Daikenchuto significantly increased the expression of *CYP3A4* mRNA at the maximum-dose 8 mg (Figures 1c and 1d). In contrast, Shosaikoto did not have a significant effect on *CYP3A4* mRNAexpression (Figure 1b).

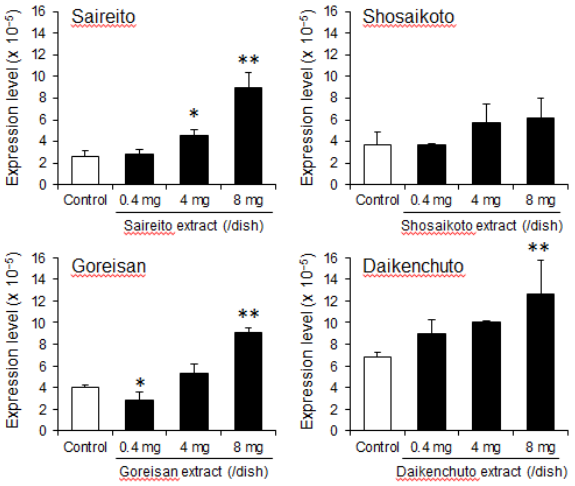

Fig 1. Impacts of Kampo medicine on the expression of *CYP3A4* mRNA in LS180 cells.
Each bar represents the mean±standard deviation (n=3).* and **Significantly different at $P<0.05$ and $P<0.01$ from the respective control, respectively (non-repeated measures analysis of variance followed by Dunnett's test).

3.2. Effects of Kampo medicine on the expression level of ABCB1 mRNA

Figure 2 shows the expression level of *ABCB1* mRNA in LS180 cells following their exposure to Saireito, Shosaikoto, Goreisan, or Daikenchuto. Saireito, Shosaikoto, and Goreisan significantly induced the expression of *ABCB1* mRNA in a dose-dependent manner in LS180 cells (Figures 2a-c). In contrast, the expression of *ABCB1* mRNA was not affected by exposure to Daikenchuto (Figure 2d).

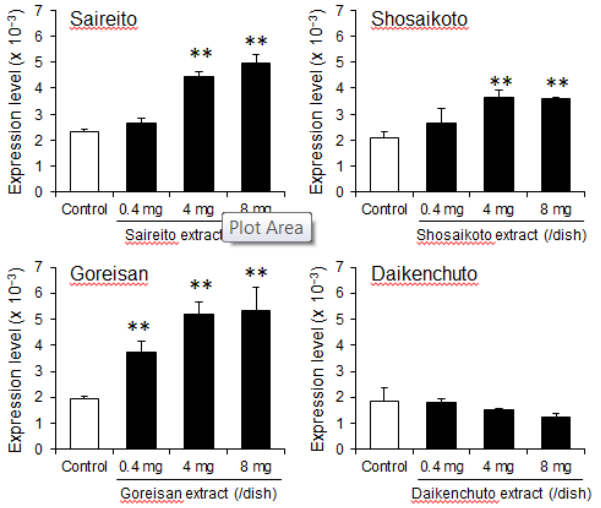

Fig 2. Impacts of Kampo medicine on the expression of *ABCB1* mRNA in LS180 cells.
Each bar represents the mean±standard deviation (n=3).**Significantly different at $P<0.01$ from the respective control (non-repeated measures analysis of variance followed by Dunnett's test).

Limited information is available on how Kampo medicine affects CYP3A4-mediated drug metabolism or ABCB1-mediated drug transport (Iwanaga *et al.*, 2010; Takiyama *et al.*, 2022). The findings of previous studies suggest that interactions between Kampo and Western medicines may occur due to the induction of CYP enzymes or ABCB1. However, serious DDIs associated with Kampo and Western medicines rarely occur, and the reason for this remains unclear. The potential mechanisms by which Kampo medicines may inhibit drug metabolism or transport are contradictory, for example, induction of CYP enzymes or ABCB1 may occur. However, little information is available regardingthe induction of CYP enzymes or ABCB1 by Kampo medicine. In this study, our results confirmed the possibility of the induction of CYP enzymes and ABCB1 by Kampo medicines.

As the exact concentration of Kampo medicine in the intestinal tract is not known, in this study, the concentration was estimated using a constant fluid volume of 250 mL (the fluid volume of a glass of water). The dose of Kampo medicine ranges from 500 to 3,000 mg per time, which means that the concentration in the intestinal tract is assumed to be approximately 2 to 12 mg/mL. Therefore, cells were exposed to Kampo medicine at concentrations of 0.4, 4, and 8 mg/mL.

The expression of *CYP3A4* mRNA increased significantly after exposure to Saireito, Goreisan, and Daikenchuto, and an increasing trend was also observed after exposure to Shosaikoto (Figure 1). Furthermore, upregulation of *ABCB1* mRNA expression was observed following exposure to Saireito, Shosaikoto, and Goreisan (Figure 2), while exposure to Daikenchuto showed no alteration in the expression of *ABCB1* mRNA. These findings indicated that different types of Kampo medicine have different induction effects, and highlight the distinct profiles of *CYP3A4* and *ABCB1* mRNA expression. Consequently, some Kampo medicines have been suggested to upregulate CYP3A4 or ABCB1 expression in enterocytes.

As Saireito consists of the ingredients included in Shosaikoto and Goreisan, the effects by Saireito may be shown by the sum of the effects of Shosaikoto and Goreisan (Table 1). However, the expression of *CYP3A4* mRNA increased significantly after exposure to Goreisan and Saireito, and exposure to Shosaikoto resulted in an increasing trend. It is possible that the ingredients of Goreisan (Alismatis rhizome, Atractylodis rhizome, *Polyporus*, and Hoelen) included the components that upregulated *CYP3A4* mRNA. In contrast, upregulation of *ABCB1*mRNA was observed after exposure to Shosaikoto, Goreisan, and Saireito, and various components were suggested to upregulate *ABCB1* mRNA.

Licorice root (kanzo), a major ingredient in Kampo medicine, has been reported to inhibit the ATPase activity of ABCB1 (Satoh et al., 2009). Additionally, Takiyama *et al.* (2022) reported that Goreisan extract has an inhibitory effect on ABCB1-mediated transport, and its ingredient alisolA inhibits CYP3A-mediated metabolism (Takiyama *et al.*, 2022). In contrast, some herbal medicines and food supplements have been reported to induce CYP1A2, CYP3A4, and ABCB1 in LS180 cells (Bramdim *et al.*, 2007). As Kampo

medicine comprises a variety of traditional herbs, it is important to identify the ingredients or components that upregulate CYP3A4 or ABCB1.

4. Conclusion

Our findings provide useful clinical information on the safety and efficacy of the combined use of Kampo and Western medicines. Furthermore, it is necessary to examine the impact of various Kampo medicines on CYP3A4 and ABCB1.

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6. References

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