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SHORT COMMUNICATION

Effect of Bakumondo-to on cytochrome P450 activities in rat liver microsomes

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KEYWORDS

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Interaction

Abstract Bakumondo-to is a traditional herbal medicine. It has been widely used for the treatment of chronic airway diseases. Recently, it was reported that several herbal medicines affected cytochrome P450 (CYP). However, there is little information about the effects of Bakumondo-to on CYP activities. In this study, we evaluated the effects of Bakumondo-to on CYP activities in rat liver microsomes. Rats were orally treated twice a day with Bakumondo-to at doses of 2.0 g/kg body weight/day for 4 days. CYP activities were determined in liver microsomes isolated from treated rats. CYP1A2, CYP2C, and CYP3A activities were measured using their specific substrates [7-methoxyresorufin, 7-methoxy-4-(trifluoromethyl)-coumarin, and 7-benzyloxyquinoline, respectively]. Bakumondo-to decreased CYP1A2 activity by 42.5 ± 7.8%, increased CYP2C activity by 158.0 ± 29.6%, and decreased CYP3A activity to 81.5 ± 7.8% of the control level. Activities were expressed as percentages of the control.

Bakumondo-to induced CYP2C activity and decreased CYP1A2 activity; it may cause drug-herbal interactions. It is suggested that combinations of Bakumondo-to and drugs that are metabolized by CYP1A2 and CYP2C should be carefully used in clinical settings.

1. Introduction

As herbal medicines tend to have minor side effects and sometimes show remarkable efficacy (Ishohama, 2001), they are an increasingly common form of complementary and alternative therapy worldwide (Al-Ramahi et al., 2015). However, a wide variety of herbal medical uses suggest the possibility for co-administration with synthetic medications, and accordingly, the potential of drug-herb interactions is high (Izzo and Ernst,

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2001). Several studies concerning drug-herb interactions were reported. St John's wort (*Hypericum perforatum*), an herbal antidepressant, is also well-known as a potent inducer of cytochrome P450 (CYP) 3A (Roby et al., 2000; Wang et al., 2001). In addition, analogs of catechin are inhibitors of CYPs, xanthine oxidase, and aldehyde oxidase (Tayama et al., 2011). Shoseiryuto, another traditional herbal medication, demonstrated CYP3A inhibitory activity in rat liver microsomes (Makino et al., 2006). *Ginkgo biloba* extract increased CYP1A2 activity and produced effects on theophylline metabolism (Tang et al., 2007). Their studies support findings that some herbal medications or supplements have potentially harmful side effects as well as adverse interactions with synthetic drugs.

Bakumondo-to, which is composed of Ophiopogon and Pinelliae tubers, Jujube, Glycyrrhiza, and Ginseng, is a traditional herbal medicine. It has been widely used for the treatment of chronic airway diseases, such as bronchitis, bronchial asthma, and cough (Saika, 1991; Sasaki, 1993; Irifune et al., 2011; Saruwatari et al., 2004). In addition, Bakumondo-to is also used for Sjögren's syndrome patients and affects their salivary secretion (Ohno et al., 1990). Clinical use of Bakumondo-to has been increasing, and it is often co-administrated with various medications, such as pranlukast, zafirlukast, or theophylline. Therefore, it is possible that Bakumondo-to interactions with synthetic drugs occur. Pranlukast is metabolized by CYP3A (Yoneda et al., 2009). Zafirlukast is metabolized by CYP2C9 and CYP3A4 (Dekhuijzen and Koopmans, 2002), and montelukast is by CYP2C8 (Backman et al., 2016). Theophylline is metabolized by CYP1A2 (Tjia et al., 1996). However, there is little information about the effect of Bakumondo-to on these activities. In this present study, we evaluated the effect of Bakumondo-to on CYP1A2, CYP2C and CYP3A activities.

As for CYP1A2 activity, it was previously investigated in human subjects. The mean activity of CYP1A2 tended to be lower after dosing in 26 healthy humans with Bakumondo-to for 7 days than that after dosing with placebo (Saruwatari et al., 2004). However the effect of the protein expression by Bakumondo-to is unclear. We also investigated the influence of Bakumondo-to on CYP protein expression.

2. Material and methods

2.1. Chemicals

Bakumondo-to extract granules for ethical use were purchased from Tsumura Co. (Tokyo, Japan). The reagents used were of the highest commercial quality available. 7-Methoxy-4-(tri fluoromethyl)-coumarin (MFC), 7-methoxyresorufin (MRF), and 7-benzyloxyquinoline (BQ) were used as an external standard for quantification of CYP2C, CYP1A2, and CYP3A activities and were purchased from BD Gentest (Woburn, MA) (Stresser et al., 2002).

2.2. Animals

Jcl:Wistar male rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). The animals were housed in cages at 22 °C with a 12-h light/dark cycle and free access to tap water and a standard pellet diet CE-2 (Clea Japan Co. Inc., Tokyo, Japan). Rats, 6 week old, were randomized into two groups (n = 3 per group) receiving either water (control) or Bakumondo-to (2.0 g/kg, p. o.) for 4 days. Their livers were taken after the rats were euthanized. The animal protocol was approved by the Animal Care and Use Committee of Hiroshima International University.

2.3. Liver preparations

Livers were excised and homogenized in four volumes of 1.15% KCl. The microsomal fraction was obtained from the homogenate by successive centrifugation at 9000g for 20 min and 105,000g for 60 min. Liver microsomes were suspended in potassium phosphate buffer. Protein concentrations were determined by the method used by Lowry et al. with bovine serum albumin as the standard protein (Lowry et al., 1951).

2.4. Assay for CYPs activities

The CYP activities were measured using the modified previous methods (Sugihara et al., 2008; Stresser et al., 2002).

CYP2C activity was assayed using MFC as the substrate. The reaction mixture containing liver microsomes (final concentration: 0.5 mg/mL), 100 μL of 5 mM NADPH, and 1840 μL of 0.1 M phosphate buffer was incubated at 37 °C for 15 min before 10 μL of 1 mM MFC was added to the incubation mixture. The fluorescent products were detected by monitoring fluorescence to determine the rates of MFC *O*-demethylation (excitation: 410 nm and emission: 510 nm). CYP1A2 activity was assayed using MRF as a substrate. The reaction mixture containing liver microsomes (final concentration: 0.5 mg/mL), 100 μL of 5 mM NADPH, and 1840 μL of 0.1 M phosphate buffer was incubated at 37 °C for 3 min before 10 μL of 1 mM MR was added to the incubation mixture. CYP1A2 activity was measured in real time by monitoring fluorescence to determine the rates of MR *O*-demethylation (excitation: 550 nm and emission: 585 nm). Rat CYP3A activity was assayed using BQ as the substrate. The reaction mixture containing liver microsomes (final concentration: 0.5 mg/mL), 100 μL of 5 mM NADPH, and 1840 μL of 0.1 M phosphate buffer was incubated at 37 °C for 10 min before 10 μL of 1 mM BQ was added to the incubation mixture. CYP3A activity was measured in real time by monitoring fluorescence to determine the rates of 7-hydroxyquinoline formation (excitation: 410 nm and emission: 510 nm).

2.5. Immunoblot analysis of CYPs

Expression levels of CYP proteins were determined by immunoblot analysis of liver microsomal proteins. The microsomal proteins (50 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10–20% polyacrylamide gel (Atto Corporation, Tokyo, Japan), and transferred to polyvinylidene difluoride (PVDF) membranes by electroblotting. Membranes were then blocked with Blocking One (Nacalai Tesque, Inc., Kyoto, Japan) for 30 min and probed with anti-rat CYP antibodies (1:2000) in 25 mM Tris-buffered saline (pH 7.6) and 0.1% Tween 20 for 24 h. The membranes were washed and antibody binding was detected with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:1000), followed by development with Chemi-Lumi One L (Nacalai Tesque, Inc., Kyoto, Japan).

2.6. Statistical analysis

Data are presented as mean ± standard deviation (S.D.). The statistical significance of differences was evaluated using *F*-test. If the *F* values were significant, the student *t*-test in homoscedasticity or Welch-*t* test in heteroscedasticity was performed. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of Bakumondo-to on CYP activity in rat liver microsomes

As shown in Fig. 1, it was observed that the MRF *O*-demethylation conversion (indicating CYP1A2 activity in the Bakumondo-to groups) showed a decrease of 42.5 ± 7.8% (% of control). The CYP1A2 activity was significantly inhibited by Bakumondo-to. MFC *O*-demethylation (indicating CYP2C activity) increased by 158.0 ± 29.6%. The activity was significantly increased compared with that of the control. 7-BQ hydroxylation (indicating CYP3A activity) was 81.5 ± 7.8% and was not significantly different from the control.

3.2. Effects of Bakumondo-to on CYP protein expression in rat liver microsomes

We evaluated the effect on CYP protein expression by Bakumondo-to (Fig. 2).

SDS–PAGE immunoblot analysis with anti-rat CYP1A2 antibodies showed that there were no significant changes in the expression of CYP1A2 proteins between control subjects and Bakumondo-to administrated subjects. In contrast, CYP2C protein expression of Bakumondo-to subjects increased to 288.9% compared with that of the control subjects. There were no significant changes in the protein expression of CYP3A.

4. Discussion

Bakumondo-to, administered for chronic airway diseases, is a herbal medicine. In general, herbal compounds, such as St John's wort and catechins, inhibit enzyme activities. However, it was unclear whether CYP activity was affected by Bakumondo-to. The aim of this study was to evaluate the effects of Bakumondo-to on CYP activity. We also examined the protein expression.

CYP3A is a major metabolic enzyme in the human liver. The enzyme metabolizes many kinds of drugs, such as midazolam, felodipine, lovastatin, and triazolam. Therefore, we evaluated the effects of Bakumondo-to on CYP3A. In this study, it was observed that Bakumondo-to had no effect on CYP3A by in rat liver microsomes. Therefore, Bakumondo-to may not interact with other drugs via CYP3A in clinical treatment.

CYP1A2 is a metabolic enzyme for theophylline, tizanidine, and caffeine. In several cases, Bakumondo-to was coadministrated with theophylline for airway inflammation and chronic bronchitis treatment. Saruwatari et al. (2004) reported that Bakumondo-to tended to inhibit the CYP1A2 activity after 7 days in an *in vivo* human study. In our study, it was observed that Bakumondo-to significantly inhibited CYP1A2

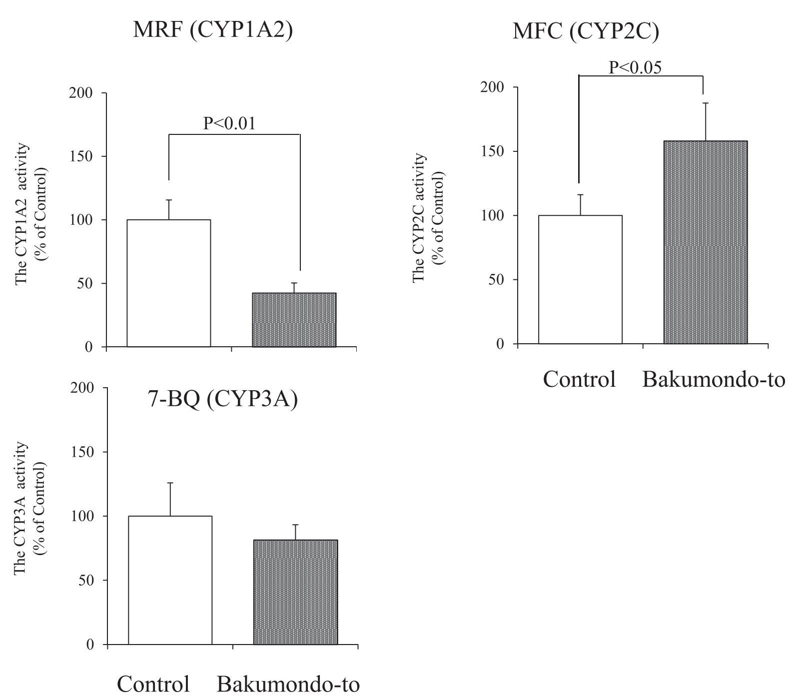

Figure 1 Changes of liver CYPs activity in rats treated with Bakumondo-to. (A) MRF: CYP1A2, (B) MFC: CYP2C, (C) BQ: CYP3A. Each CYPs activity was measured in real time by monitoring fluorescence to determine the rates of moderate substrate formation. Each bar represents mean ± S.D. of three experiments.

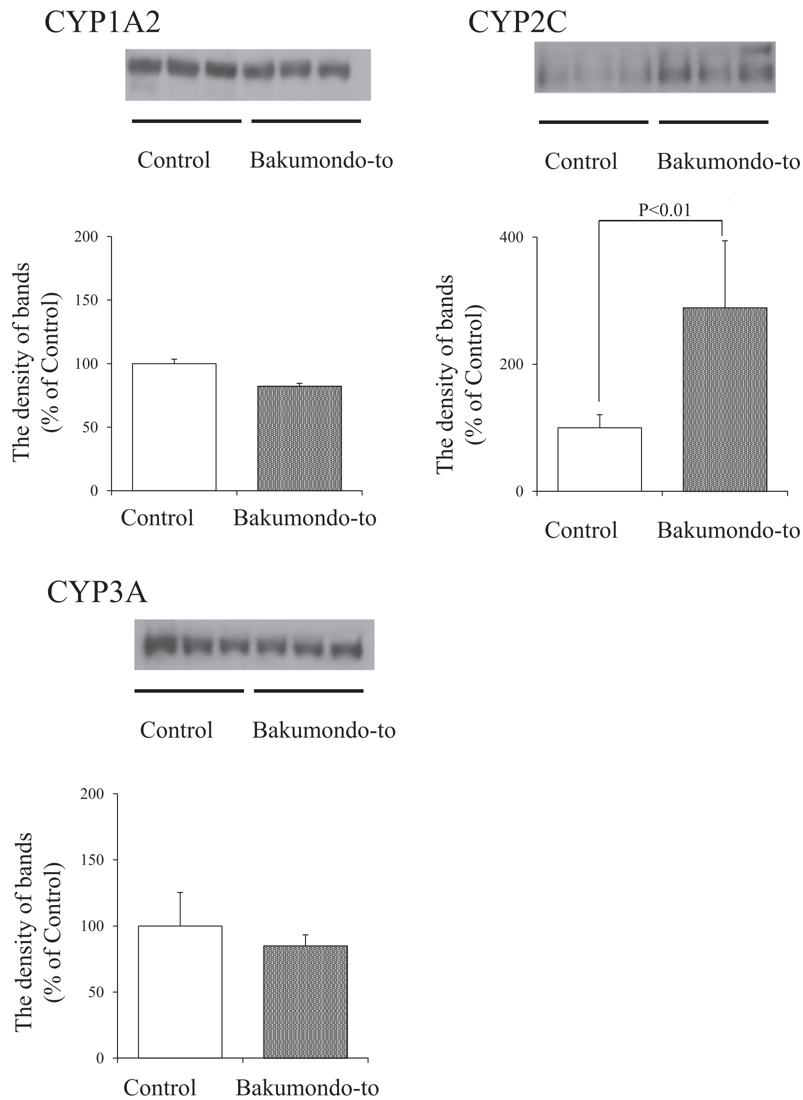

Figure 2 Analysis of the expression and the bands density of liver CYPs protein in rats treated with Bakumondo-to by Western immunoblotting with (A) anti-CYP1A2 antibody, (B) anti-CYP2C9 antibody, and (C) anti-CYP3A antibody. Each point represents the mean ± S.D. of three experiments.

activity, but its protein expression was not changed compared with that of the control. It suggested that the inhibition of CYP1A2 activities by Bakumondo-to is independent of protein expression. Taking our and Saruwatari's study data into account, Bakumondo-to may change the affinity of CYP1A2. Chang reported that Ginseng root extract, one of the components of Bakumondo-to, inhibited CYP1A2 activity (Chang et al., 2002). However, the inhibitory components and mechanisms are still unknown and need to be clarified.

CYP2C is approximately 25% of P450 in human liver microsomes (Imaoka et al., 1996). It is reported that CYP2C is the key enzyme for the metabolism of zafirlukast, montelukast, omeprazole, and phenytoin (Dekhuijzen and Koopmans, 2002; Karonen et al., 2012). Zafirlukast or montelukast, a leukotriene receptor antagonist, is used for the treatment of asthma and allergic rhinitis. Bakumondo-to is co-administrated with zafirlukast or montelukast in several cases. Therefore, we also examined the effect of Bakumondo-to on CYP2C. The activities were increased by 158%, and the density of CYP2C bands were increased by 288% compared with that of the control groups. The data suggest that the increased activities depend on the induced protein expression. Only 4 days of treatment caused CYP2C induction. If Bakumondo-to is administered for long periods in clinical situations caution should be taken to prevent drug-herbal interactions via CYP2C.

5. Conclusion

In conclusion, Bakumondo-to induced CYP2C activities, followed by protein expression. In contrast, Bakumondo-to decreased CYP1A2 activity which may be caused by the drug-herbal interaction. Therefore it is advised to carefully use combinations of Bakumondo-to with drugs that are metabolized by CYP1A2 and CYP2C in clinical settings.

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