Ganoderic acid B's influence towards the therapeutic window of trifluoperazine (TFP)

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Abstract

Background: Ganoderic acid B is an important bioactive ingredient isolated from Ganoderma lucidum, and exhibits various pharmacological activities.

Aims: To investigate the influence of Ganoderic acid B towards the therapeutic window of trifluoperazine (TFP).

Methods: In vitro human liver microsomes (HLMs) incubation system was used to determine the inhibition of Ganoderic acid B towards the glucuronidation of trifluoperazine (TFP).

Results: Ganoderic acid B exerted concentration-dependent inhibition towards the glucuronidation of TFP. Furthermore, Dixon plot was used to determine the inhibition type. The intersection point was located in the second quadrant in Dixon plot, indicating the competitive inhibition of Ganoderic acid B towards TFP glucuronidation. Through fitting the data using competitive nonlinear fitting equation, the inhibition kinetic parameter was calculated to be 56.7 uM.

Conclusion: All this data indicated the potential influence of Ganoderic acid B-containing herbs towards therapeutic window of TFP. Given that the glucuronidation reaction of TFP is the probe reaction of UGT1A4, the data obtained from the present study also indicated the potential influence of Ganoderic acid-containing herbs towards the therapeutic window of drugs mainly undergoing UGT1A4-mediated metabolism.

Keywords: Ganoderic acid B, trifluoperazine (TFP), UDP-glucuronosyltransferase (UGT) 1A4
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Introduction

Ganoderma lucidum, a well-known traditional Chinese medicine, has been utilized for longevity and treatment of multiple diseases in Asia for many years[1]. Ganoderic acids, the oxygenated lanostane-type triterpenoids isolated from Ganoderma lucidum, have been reported to exhibit multiple biochemical and pharmacological activities, such as anti-tumor[2,3], cardioprotective and liver protection activity[4,5]. The development of the compounds needs the adverse effects and in vivo elimination information, besides the pharmacologically therapeutic role.

Metabolic enzymes-catalyzed biotransformation process has been considered to be an important cause for the adverse effects of drugs. For example, cytochrome P450 (CYP) 2E1-mediated metabolic activation of acetaminophen has been regarded as the major reason for acetaminophen-induced acute liver failure[6]. Additionally, the inhibition of compounds towards the activity of drug-metabolizing enzymes (DMEs) is another reason for drugs-induced adverse effects. For example, the anti-tumor drug noscapine exhibits significant inhibitory effects towards CYP3A4 and CYP2C9-catalyzed warfarin metabolism, and then induces clinical noscapine-warfarin interaction[7]. For one of the most important phase II DMEs UDP- glucuronosyltransferases (UGTs), the activity inhibition will also induce the severe adverse effects. For example, HIV therapeutic drug indinavir inhibited UGT1A1-mediated bilirubin glucuronidation to induce the elevation of unconjugated bilirubin[8].

Trifluoperazine (TFP) is a typical antipsychotic of the phenothiazine chemical class, and its major clinical application is to treat schizophrenia[9]. The adverse effects of TFP contain extrapyramidal reactions (e.g., Parkinson-like symptoms, dystonia, etc.), drowsiness, fatigue, muscular weakness, and hypotension.

According to the reports from Drugs.com website, a total of 1056 drugs can induce the drug-drug interaction (DDI) with TFP. TFP has relatively narrow therapeutic window, and major drug-metabolizing enzyme to contribute to the metabolism of TFP is UGT1A4. The present study aims to investigate the inhibition of Ganoderic Acid B towards the metabolism of TFP.

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Materials and Methods

Chemicals and Reagents

Tris-HCl, 7-hydroxycoumarin, and uridine-5-diphosphoglucuronic acid (UDPGA) (trisodium salt) were obtained from Sigma-Aldrich (St. Louis, MO). Sigma-Aldrich also offers the trifluoperazine dihydrochloride for the research needs. Pooled human liver microsomes (HLMs) were prepared according to previous reports[10,11].

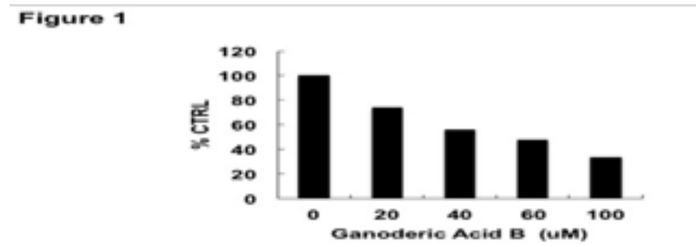
Ganoderic Acid B's inhibition towards the glucuronidation of TFP

The formation of TFP glucuronide and the chromatography conditions were carried out as previously described[12]. In brief, the incubation system (200 uL) contains 50 mM Tris-HCl (pH=7.4), 25 ug/mL lamethicin (from Trichoderma viride), 0.1 mg/ml HLMs, 5 mM MgCl2, 5 mM UDPGA, and various concentrations of TFP and Gaboderic Acid B. The incubation time was 20 min, and the incubation temperature was 37^{0}C. The incubation conditions ensure the linear reaction of TFP glucuronidation. The reaction was terminated with the equal volume of methanol, and the aliquots (10 uL) were used for analysis. The inhibition kinetic type was determined through Dixon plot (Reaction velocity versus the concentrations of Ganoderic acid B), and the inhibition kinetic parameters were calculated using the nonlinear regression equations as followed according to previous literatures13-15. $V=(VmaxS)/(Km(1+I/Ki)+S)$ (1) $V=(VmaxS)/(Km+S)(1+I/Ki)$ (2)
The equations (1) and (2) were employed for competitive and noncompetitive fitting, respectively. The terms in the equations were defined as followed: V is the velocity, S is the concentration of substrate, Km is the metabolism kinetic parameter, I is the concentration of inhibitor, and Ki is the inhibition kinetic parameter.

Results

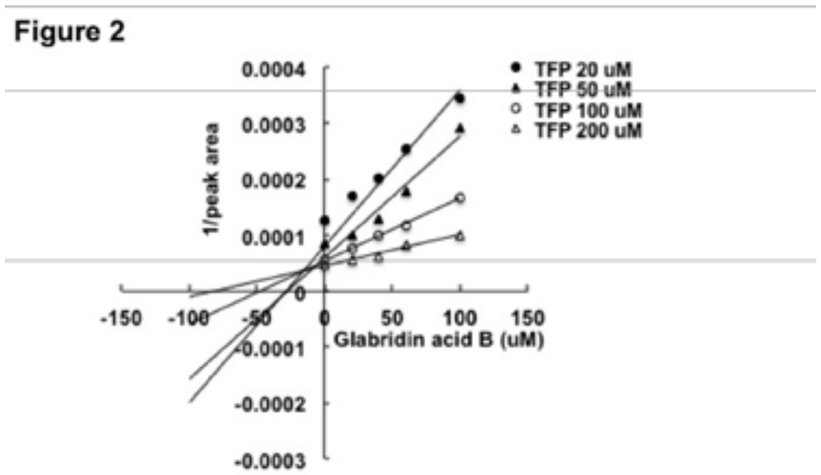
Multiple concentrations of Ganoderic acid B were used to screen the inhibition potential of Ganoderic acid B towards TFP glucuronidation, and the results showed that 0, 20, 40, 60 and 100 uM of Ganoderic acid B inhibited the glucuronidation activity of TFP by 0, 26.6%, 44.1%, 52.9%, and 66.9%, respectively (Fig. 1).

Fig. 1 Concentration-dependent inhibition of Ganoderic Acid B towards the glucuronidation of trifluoperazine (TFP). The residul percentage of control activity was employed to represent the inhibition capability of Ganoderic acid B towards the glucuronidation activity of TFP. Two replicate experiments were carried out, and the values represent the mean value of duplicate experiment.



Furthermore, the Dixon plot using the 1/reaction velocity versus the concentrations of Ganoderic acid B was drawn to determine the inhibition kinetic type, and the intersection point was located into the second quadrant, indicating the competitive inhibition type (Fig. 2). Through fitting the data using competitive nonlinear fitting equation, the inhibition kinetic parameter (Ki) was calculated to be 56.7 uM.

Fig. 2 Determination of inhibition kinetic type using the Dixon plot. The Dixon plot used the reaction velocity versus the concentrations of Ganoderic acid B. Each data point represents the mean of the duplicate experiments.



Discussion

In vitro incubation system is an useful tool to investigate the metabolic behavior of xenobiotics and metabolism-mediated adverse effects. Through adding different co-factors, the phase I and phase II metabolic pathways can be well separated, and the inhibition behavior towards the metabolism can be studied through co-incubation with another compound. The present study investigated the inhibition potential of Ganoderic acid B towards TFP glucuronidation, and the competitive inhibition of Ganoderic acid B was demonstrated, indicating the utilization risk when co-administration of Ganoderic acid B-containing herbs and TFP. Previous literatures have shown that other herbal components also exhibited inhibitory effects towards the glucuronidation of TFP[16]. Therefore, much attention should be paid to the clinical co-utilization of TFP and Ganoderic acid B-containing herbs.

It should be noted that the glucuronidation reaction of TFP has been widely employed as the probe reaction of UGT1A4. Many endogenous and xenobiotic compounds have been demonstrated to be the good substrates of UGT1A4. For example, UGT1A4 can conjugate 25-hydroxyvitamin D3 (25OHD3) which is a clinical biomarker for assessment of vitamin D status[17]. UGT1A4 majorly contributed to the glucuronidation of tacrolimus which is a potent immunosuppressant[18]. UGT1A4 also catalyzed the metabolic elimination of primary, secondary, and tertiary amines, carcinogenic aromatic amines (β-naphthylamine, 4- aminobiphenyl, and benzidine), androgens, progestins, and plant steroids (hecogenin, diosgenin, and tigogenin)[19,20]. When Ganoderic acid B-containing herbs were co-administered with these drugs, the potential influence towards the therapeutic window of these drugs might occur.

Conclusion

The potential influence of Ganoderic acid B-containing herbs towards the therapeutic window of trifluoperazine (TFP) was reported in the present study, as indicated by the in vitro inhibition of Ganoderic acid B towards the glucuronidation of TFP. Additionally, many compounds with similar structures with Ganoderic acid B are speculated to be the inhibitors of TFP glucuronidation, and will be investigated in the future.

Acknowledgement

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