Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense*)
Lat. Am. J. Pharm. **32** (8): 1252-4 (2013)

Short communication
Received: July 21, 2013
Revised version: July 27, 2013
Accepted: July 28, 2013

Glucoaurantio-Obtusin Deglycosylation Biotransformation Process Strongly Influences the Clinical Safety of Hypnotic Agent Propofol

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SUMMARY. **Drug-drug interaction and herb-drug interaction have been frequently reported when patients take propofol in combination with other important clinical drugs or herbs. Intestinal bacteria-catalyzed biotransformation process might significantly change the properties of compounds, such as the efficiency, toxicity, and the pharmacokinetic behaviours. The present study aims to investigate whether the intestinal bacteria that catalyzed biotransformation of glucoaurantio-obtusin can influence the inhibitory capability towards the metabolism of the hypnotic agent, and propofol was selected as the example. The results showed that aurantio-obtusin exhibited stronger inhibition than glucoaurantio-obtusin towards propofol glucuronidation at various tested concentrations. Furthermore, the noncompetitive inhibition of aurantio-obtusin towards propofol glucuronidation was demonstrated, and the inhibition parameter (K_i) was calculated to be 78.7 μM. All these information will be helpful to understand the clinical safety when propofol is co-administered with herbs.**

INTRODUCTION

Hypnotic agents are a class of psychoactives able to induce sleep, and have been widely employed for the treatment of insomnia, and in surgical anesthesia. Examples of this kind of drugs are amobarbital, pentobarbital, triazolam, zopiclone, and propofol [1]. Numerous drug-drug interactions have been reported between hypnotic drugs and other clinical drugs. For example, the hypnotic drug triazolam is metabolized by CYP3A, and the co-administered drug itraconazole can significantly increase the plasma concentration of triazolam through inhibition of its metabolism catalyzed by CYP3A4 [2]. Administration of pentobarbital can significantly induce the activity of CYP1A2 which catalyzed many clinical drugs [3].

The utilization of herbs has been becoming more and more popular due to its low adverse effect, which is in part attributed to the detoxification functioned by intestinal bacteria. Besides the metabolism of some endogenous substances (*e.g.* bile acids), intestinal bacteria also plays a key role in the biotransformation of xenobiotics, such as the deglycosylation process of some herbs (*e.g.* ginsenosides, etc.). This kind of biotransformation process can sometimes completely change the structures of compounds, which furtherly affect their properties, including efficiency, toxicity, and pharmacokinetics.

The present study aims to investigate whether the deglycosylation biotransformation process of herbal ingredients can affect the metabolic behaviour of hypnotic agents. Glucoaurantio-obtusin was selected as the representative herbal ingredient, and propofol was chosen as the representative hypnotic drug.

MATERIALS AND METHODS

Chemicals

Glucoaurantio-obtusin (purity ≥ 98%) and aurantio-obtusin (purity ≥ 98%) were purchased

KEY WORDS: Deglycosylation biotransformation, Drug-herb interaction, Hypnotic agent, Propofol.

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ISSN 0326-2383

from Sichuan Weikeqi Biological Technology Co., Ltd. Propofol, 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine-5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St Louis, MO). The pooled human liver microsomes (HLMs) was purchased from BD Biosciences. Acetonitrile and methanol with HPLC grade was purchased from Merck, and all aqueous solutions were prepared using ultrapure Milli-Q water (>18 MΩ). All other reagents were of HPLC grade or of the highest grade commercially available.

Inhibition of glucoaurantio-obtusin and aurantio-obtusin towards propofol glucuronidation reaction

The incubation system and reaction condition for propofol glucuronidation were the same as previously described [4]. Dose-dependent inhibition of aurantio-obtusin towards the glucuronidation of propofol was investigated due to stronger inhibition of aurantio-obtusin towards propofol glucuronidation. The reaction velocity of propofol glucuronide was determined at various concentrations of propofol and aurantio-obtusin. Furthermore, the data fitting was performed with Dixon plot (the reaction velocity versus the concentrations of aurantio-obtusin) and Lineweaver-Burk plot (the reaction velocity versus the concentrations of propofol).

Nonlinear regression was used to calcultae the inhibition kinetic parameters (K_i) according to the equations for competitive inhibition [**1**] and noncompetitive inhibition [**2**].

$$V = V_{max}*[S]/(K_m/(1+([I]/K_i))+[S]) \quad [\mathbf{1}]$$

$$V = V_{max}*[S]/(K_m+[S])*(1+([I]/K_i)) \quad [\mathbf{2}]$$

where the items are defined as followed: V is the reaction velocity, $[S]$ and $[I]$ are the concentrations of substrate and inhibitor, respectively. K_m value is the substrate concentration in which the velocity reached to half of the maximum velocity (V_{max}) of the reaction. K_i value is the inhibition constant.

RESULTS

The residual activity of propofol glucuronidation at different concentration of glucoaurantio-obtusin and aurantio-obtusin is shown in Fig. 1. The residual activity of glucuronidation activity was 87.5, 88.2, 86.5, 78.5,

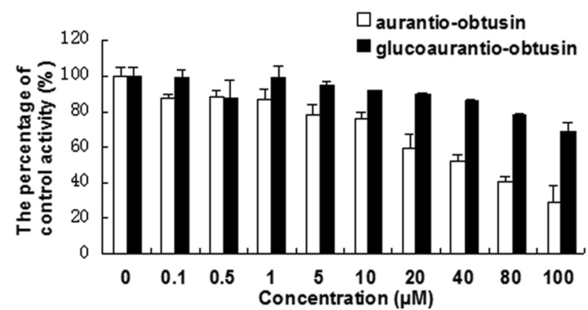

Figure 1. Residual activity of propofol glucuronidation at different concentration of glucoaurantio-obtusin and aurantio-obtusin.

76.1, 59.6, 51.8, 41.0, and 29.0% for 0.1, 0.5, 1, 5, 10, 20, 40, 80, and 100 μM of aurantio-obtusin. Compared with the inhibition capability of aurantio-obtusin, the lower inhibition potential of glucoaurantio-obtusin towards propofol glucuronidation was observed, with the residual activity to be 98.8, 87.4, 98.8, 94.9, 92.1, 89.7, 86.1, 78.1, and 68.4 for 0.1, 0.5, 1,5, 10, 20, 40, 80, and 100 μM of glucoaurantio-obtusin. The intersection point in Dixon plot and Lineweaver-Burk plot can reflect the inhibition type. If the intersection point was located in verticle axis in Dixon plot and the second quadrant in Lineweaver-Burk plot, the inhibition type belongs to be competitive inhibition. The intersection point in noncompetitive inhibition was located in horizontal axis Dixon plot and Lineweaver-Burk plot. The Dixon plot (Fig. 2) and Lineweaver-Burk plot (Fig. 3) demonstrated the noncompetitive inhibition of aurantio-obtusin towards the glucuronidation of propofol. Through nonlinear regression with noncompetitive inhibition equation, the inhibition kinetic parameter (K_i) was calculated to be 78.7 μM.

DISCUSSION

Propofol is an important hypnotic agent widely used in clinic, and marketed as Diprivan by AstraZeneca. Propofol has narrow therapeutic index, and the slight alteration of serum concentration of propofol might induce the toxicity [4]. Many herbal ingredients have been reported to exert inhibition towards the glucuronidation of propofol, such as liquiritigenin [4], cryptotanshinone, and dihydrotanshinone I [5].

When investigating the metabolism behaviour of compounds, *in vivo* model might be very complex due to its difficulty to seperate the phase I metabolism from phase II metabolism.

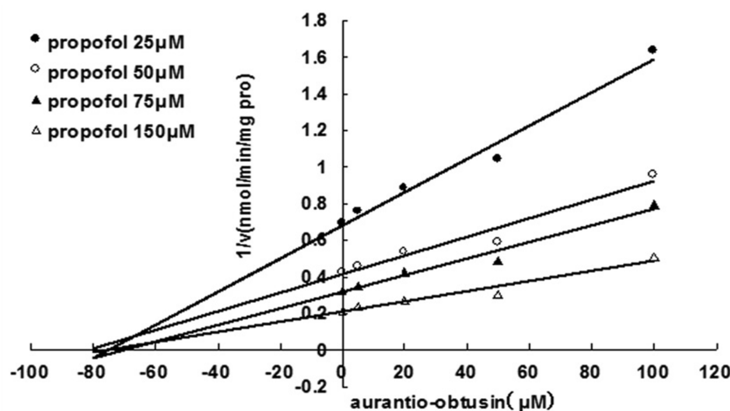

Figure 2. Dixon plot showing the noncompetitive inhibition of aurantio-obtusin towards the glucuronidation of propofol.

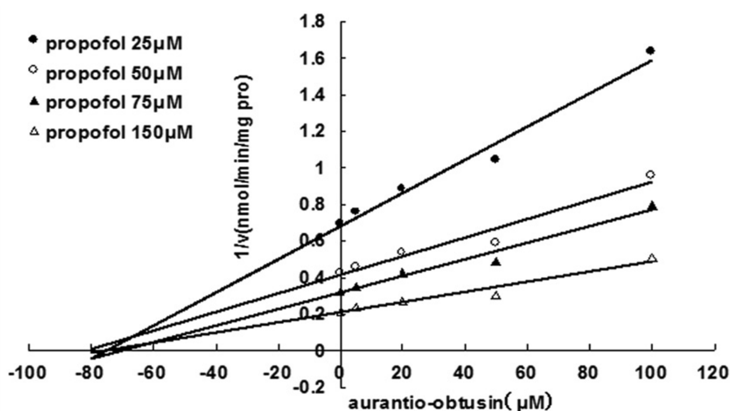

Figure 3. Lineweaver-Burk plot showing the noncompetitive inhibition of aurantio-obtusin towards the glucuronidation of propofol.

Therefore, *in vitro* models can be more suitable for the study of compounds' inhibition towards the glucuronidation of propofol. Compared with hepatocytes system, human liver microsomes incubation systems are easier to construct the phase I and phase II metabolic system through adding the corresponding co-factors including NADPH and UDPGA [6-8].

In this study, we utilized *in vitro* incubation mixture to evaluate the inhibition of propofol glucuronidation by two important herbal ingredients, glucoaurantio-obtusin and aurantio-obtusin, trying to indicate the important role of intestine bacteria-mediated deglycosylation biotransformation process in the inhibitory potential towards propofol glucuronidation. The results indicated that the deglycosylation of glucoaurantio-obtusin can significantly increase the inhibition potential towards the glucuronidation of propofol. Additionally, the inhibition kinetic information (including inhibition type and parameters) was furtherly determined for the inhibition of aurantio-obtusin towards propofol glucuronidation, which will be helpful for deep understanding of the clinical safety when co-administration with propofol and herbs. Additionally, because the human liver microsomes (HLMs)-catalyzed propofol has been widely used as the probe reaction for phenotyping UGT1A9, necessary clinical monitoring is needed when the herbs containing aurantio-obtusin and/or glucoaurantio-obtusin was co-administered with the drugs mainly undergoing UGT1A9-catalyzed metabolism.

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