

***In vitro* evidence of possible influence of scutellarein towards bile acids' metabolism**

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Abstract

Background: The glucuronidation process has been regarded as the key elimination process for toxic bile acids. UDP-glucuronosyltransferase (UGT) 1A3 is one important metabolizing enzyme involved in this process.

Objective: To evaluate the inhibition of UGT1A3 by scutellarein which is an important herbal ingredient using *in vitro* method, trying to indicate the possibility of toxicity due to the accumulation of toxic bile acids.

Methods: Due to the difficulty to gain the standards of biles acids' glucuronides, the recombinant UGT1A3-catalyzed 4-methylumbelliferone (4-MU) glucuronidation reaction was employed to profile the activity of UGT1A3.

Results: The results showed that scutellarein inhibited UGT1A3 in a concentration-dependent behaviour. Competitive inhibition was demonstrated using both Dixon plot and Lineweaver-Burk plot, and the inhibition kinetic parameter (K_i) was calculated to be 5.8 μM.

Conclusion: All these data reminded the necessary monitoring of the levels of bile acids in plasma when utilizing scutellarein and the herbs containing this compound.

Key words: scutellarein, bile acids metabolism, *in vitro* methods

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Introduction

Bile acids (BAs), synthesized from cholesterol via a process catalyzed by at least 14 enzymes in liver, play a key role in the absorption of triglycerides, cholesterol, and lipid-soluble vitamins¹. Due to their detergent properties, the accumulation of BAs exerted inherent cytotoxicity. The metabolic alterations through glucuronidation conjugation reaction can efficiently weaken the toxicity of BAs due to the detoxification properties of glucuronidation pathways². Glucuronidation process often occurs in the 3-hydroxyl group or the 24-carboxyl group to form ether- or acyl-type

glucuronides^{3,4}. UDP-glucuronosyltransferases (UGTs) are the important metabolizing enzymes involved in the glucuronidation of BAs. For example, human UGT1A3 has been demonstrated to catalyze the glucuronidation at the 24-carboxyl group of lithocholic acid (LCA) in liver, and UGT2B4 and UGT2B7 play a key role in the glucuronidation elimination of hyodeoxycholic acid, and 6Q-hydroxylated metabolite of LCA^{5,6}.

The inhibition of the activity of UGT isoforms involved in the glucuronidation elimination of bile acids might damage the detoxification process of bile acids. With the increasing utilization of herbs, the components existed in herbs might have the potential to inhibit the major UGT isoforms participating in the metabolism of bile acids.

This study investigated the inhibition of herbal ingredient scutellarein towards one important UGT isoform involved in the metabolism of bile acid, UGT1A3. Given that it is difficult to get the standards of bile acids' metabolites, the recombinant UGT1A3-catalyzed 4-methylumbelliferone

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glucuronidation reaction was used in the present study.

Methods

Chemicals

Scutellarein (purity >98%), 4-methylumbelliferone (4-MU), 4-methylumbelliferone-beta-D-glucuronide (4-MUG), and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were obtained from Sigma-Aldrich (St Louis, MO). HPLC grade acetonitrile was obtained from Merck, and all aqueous solutions were prepared using ultrapure Milli-Q water (>18 M Ω). Recombinant UGT1A3 was obtained from BD Gentest Corp. (Woburn, MA, USA).

In vitro evaluation of scutellarein's inhibition towards UGT1A3 activity

The activity of UGT1A3 was phenotyped using recombinant UGT1A3-catalyzed 4-MU glucuronidation probe reaction as previously described⁷. To obtain more inhibition kinetic

information, various concentrations of scutellarein and 4-MU were used, and Dixon plot and Lineweaver-Burk plot were employed to determine the inhibition kinetic type.

Results

Instead of the selection of bile acids' glucuronidation, the present study used the 4-MU glucuronidation as the representative reaction to phenotype the activity of UGT1A3 which has been reported to be the main metabolizing enzymes participating the detoxification process of bile acids. Concentration-dependent inhibition of scutellarein towards UGT1A3 was demonstrated in the present study (figure 1). Both Dixon plot (figure 2A) and Lineweaver-Burk plot (figure 2B) demonstrated the competitive inhibition of scutellarein towards UGT1A3. The inhibition kinetic parameter (K_i) was calculated using the second plot with the slopes from the Lineweaver-Burk plot versus the concentrations of scutellarein, and the value was 5.8 μ M (figure 3).

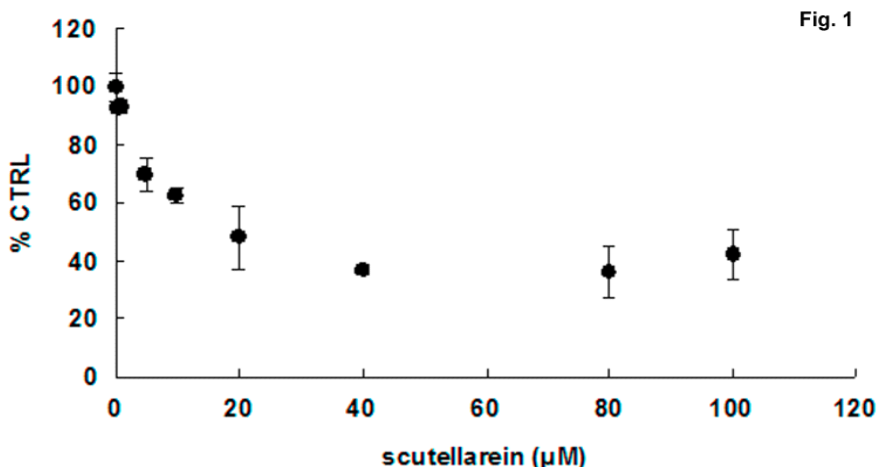


Figure 1: The dose-dependent inhibition behaviour of scutellarein towards UGT1A3 activity. The experiments were carried out for triplicate, and the data were given as mean plus standard deviation (S.D.)

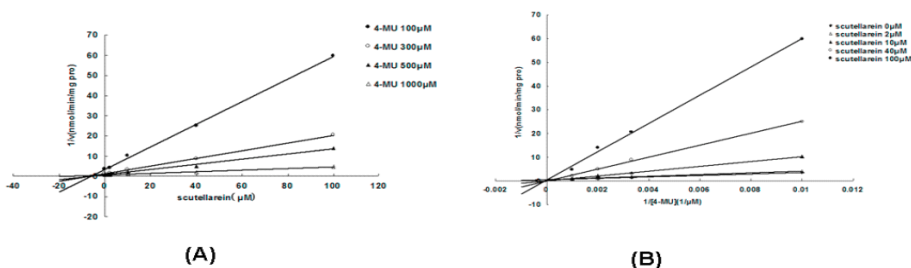


Fig. 2

Figure 2: Determination of inhibition kinetic type using Dixon plot (A) and Lineweaver-Burk plot (B). Each point represents the mean value of two replicates.

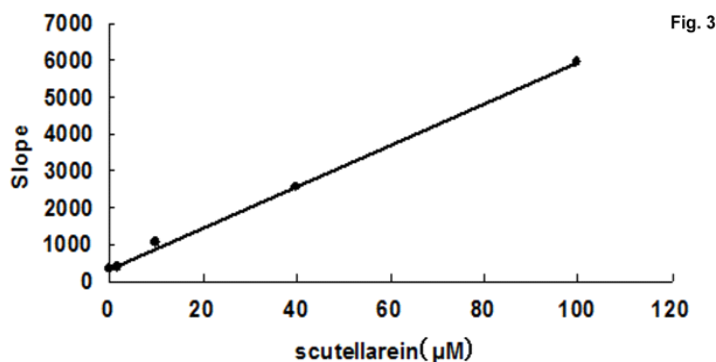


Fig. 3

Figure 3: Determination of the inhibition parameter (K_i) using second plot with the slopes from Lineweaver-Burk plots towards the concentrations of scutellarein

Discussion

Xenobiotics might induce the perturbation of bile acids homeostasis. For example, the present studies carried out by Yamazaki *et al.* investigated the influence of 13 hepatotoxins-induced liver injury towards the levels of bile acids, including acetaminophen, bendazac, cyclosporine A, carbon tetrachloride, ethionine, methapyrilene, naphthylisothiocyanate, tetracycline, ticlopidine, carbamazepine, chlorzoxasone, flutamide, and nimesulide⁸. The results showed that the selected bile acids in the circulation can be affected. The influence towards the bile acids homeostasis might be through various mechanisms. Xenobiotics can affect the activity of cytochrome P450 7a1 which is the rate-limiting enzyme in the synthesis of bile acid from cholesterol⁹. Additionally, xenobiotics can also affect the bile acids homeostasis through affecting the functions of some nuclear receptors, such as farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor (VDR), and peroxisome proliferator-activated receptor (PPAR)¹⁰⁻¹³.

In the present study, the inhibition of main metabolizing enzyme involved in the metabolic elimination of bile acids UGT1A3 by scutellarein was demonstrated, which might influence the elimination of bile acids through glucuronidation process. That indicated us the necessary monitoring of bile acids level in serum when taking scutellarein and the herbs containing this compound. It should be noted that 4-MU was used as a probe substrate in the present study to investigate the inhibition of scutellarein towards UGT1A3-catalyzed bile acids glucuronidation, which might result in the substrate-dependent inhibition.

Conclusion

The inhibitory effect of scutellarein towards the major metabolizing enzyme of bile acids provides the risk of damage of bile acids balance.

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