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ABSTRACT

IN-VITRO MECHANISTIC STUDIES ON THE INTESTINAL ABSORPTION AND FIRST-PASS METABOLISM OF SCUTELLAREN AND SCUTELLARIN

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Purpose: Scutellarin, which belongs to flavonoid glycoside, is a well-known medicine to treat cardiovascular disease. It was reported that the oral absorption of scutellarin in rat is very low (<5%). Previous studies suggested that scutellarin needs to be hydrolyzed into its aglycone scutellarein by bacterial enzymes before being absorbed into vivo. A new mono-glucuronidation conjugate (iso-scutellarin) was found in the human plasma after oral administration of scutellarin. To better elucidate above in-vivo findings, the present study aims to elucidate the intestinal transport mechanism as well as the role of glucuronidation of scutellarein in both intestine and liver *in vitro,* which will help to uncover the factors that contribute to its limited oral absorption.

Methods: The bi-directional transports of scutellarein and scutellarin were studied in the Caco-2 cell monolayer and quantified by HPLC/UV. Glucuronidation was investigated by incubation scutellarein or scutellarin with human liver microsomes (HLM), human intestine microsomes (HIM) and UGTs. The generated metabolites were identified by LC/MS/MS and quantified by HPLC/UV. Metabolic kinetics parameters including K_m, V_{max} and C_{lint} were obtained by fitting the data to the typical Michaelis-Menten equation.

Results: No significant efflux were observed in the bi-directional transports of scutellarein and scutellarin with permeability coefficient (P_{app}) of $7.9\pm1.5\times10^{-6}$ cm/s (AP to BL) and $13\pm1.2\times10^{-6}$ cm/s (BL to AP) for scutellarin; $12.6\pm1.3\times10^{-6}$ cm/s (AP to BL) and $14.3\pm0.3\times10^{-6}$ cm/s (BL to AP) for scutellarein, suggesting that scutellarein and scutellarin are mainly transported via transcellular pathway by passive diffusion. As the permeability of scutellarein is about 2 times than those of scutellarin, so it was considered as the predominant form that was absorbed in the small intestine. The major glucuronidation metabolite in Caco-2 cell monolayer model, HLM and HIM was identified as the scutellarin. And a new metabolite, which has the same molecular weight but different retention time as that of scutellarin, was detected from the bi-direction transport studies on Caco-2 monolayer. The V_{max} and K_m of glucuronidation of scutellarein into scutellarin in HLM is 3.08±0.23 nmol/min/mg and 9.96±3.11 μM, respectively. The biotransformation of scutellarein to scutellarin was extensive in human liver microsomes and human intestine microsomes with intrinsic clearances (V_{max}/K_m) of 309 and 46 μl/min/mg, respectively. Further enzyme kinetic studies using human recombinant glucuronsyltransferases (UGT) isozymes showed that UGT1A1, UGT1A3, and UGT1A9 were the major isoenzymes contributing to the glucuronidation of scutellarein, whereas UGT1A6, UGT1A7, UGT1A8 and UGT2B15 were less efficient with lower kinetic profiles.

Conclusion: In summary, scutellarein and scutellarin could be readily passed across the Caco-2 cell monolayer by passive diffusion. Scutellarein with the higher apparent permeability coefficient was considered as the predominant form being absorbed into the small intestine. Scutellarein is subjected to extensive liver and intestine glucuronidation resulting in low oral bioavailability. The glucuronidation of scutellarein is predominant catalyzed by UGT1A1, UGT1A3, and UGT1A9 into the mono-glucuronidation conjugate in human intestine and liver.