Major Anthraquinones Derived from Polygoni Multiflori Radix Alter Bile Acid Disposition in Sandwich Cultured Rat Hepatocytes

L. Kang, Y. Wu, S. He, J. Rao, W. Zhu, Y. Guan, D. Li, J. Xu, G. Li, L. Si, J. Huang
Huazhong University of Science and Technology

Purpose
Herbal medicines have been widely used for the treatment of a variety of diseases, the general use of such medicines, however, raises medical concerns regarding their adverse effects. Hepatic adverse reaction associated with Polygoni Multiflori Radix (PMR) has been frequently reported in recent years. The major chemical components in PMR including flavonoids, polyphenols, stilbenes, and anthraquinones (AQs), exhibit diverse pharmacological and toxicological effects. Highly-enriched AQs, such as emodin, chrysophanol and physcion, were also found to be hepatotoxic. The aim of the present study was to investigate the effect of individual AQs on the disposition of endogenous and exogenous bile acids (BAs) using sandwich cultured rat hepatocytes.

Methods
All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of Tongji Medical College. Rat hepatocytes were isolated according to a two-step collagenase digestion method. Sandwich configuration was established by overlaying cells with 0.25 mg/ml Matrigel followed by culture for another three days. Cytotoxicity was assessed in SCRHs using MTT colorimetric assay and LDH assay kit, respectively. Intracellular TBA level was measured by TBA assay kit. On day 4 of culture, SCRHs were pre-incubated with 5, 25, and 50 microM emodin, chrysophanol or physcion in the presence (cells + bile) or absence (cells) of Ca2+/Mg2+ for 15 min. Subsequently, the hepatocytes were coincubated for another 15 min at 37 degree Celsius with standard HBSS containing probe substrates (1 microM d5-TCA, or 10 microM CDFDA) in the presence of individual AQs (0, 5, 25, or 50 microM). The intracellular concentration of d5-TCA was measured by LC-MS/MS, while lysate levels of CDF were determined by spectrofluorimetry. Moreover, accumulation of endogenous bile acids in SCRHs was examined by LC-MS/MS. In addition, basal efflux of d5-TCA in SCRHs, together with uptake of d5-TCA in primary rat hepatocytes, was investigated to elucidate the role of AQs on basolateral efflux and uptake transporters. Quantitative real-time PCR and western blotting assays were employed to study the impact of AQs on BA transporters and enzymes involved in BA disposition.

Results
MTT and LDH release assays demonstrated all three AQs of interest were not toxic at concentrations ≤ 50 microM. Interestingly, intracellular accumulation of TBA was significantly increased after treatment of each AQ at concentrations higher than 25 microM. Moreover, Intracellular concentrations of specific endogenous BAs, such as TCA, TMCA, and GMCA, were significantly elevated by all three AQs. Emodin and chrysophanol significantly inhibited bile salt export pump and multidrug resistance-associated protein 2 (Mrp2), respectively, as evidenced by decreased biliary excretion index (BEI) of d5-TCA and CDF. Furthermore, all three AQs reduced the basal efflux of d5-TCA, while emodin but not chrysophanol and physcion decreased d5-TCA uptake in Na+-containing HBSS, indicating an inhibitory effect on rat Ntcp. In addition, gene levels of Mrp2, Mrp3 and Mdr2 were down-regulated by emodin and physcion, while protein levels of Mrp2, Mrp3 and Mrp4 were strongly suppressed by emodin. To be noted, subsequent adaptive gene regulation alleviated, to a certain extent, but not prevent from toxic BA accumulation.

Conclusion
Impaired BA canalicular transport may directly result in hepatotoxicity of PMR derived AQs. Moreover, inhibition on basolateral efflux transporters exacerbated BA retention. Down-regulation of Mrps by AQs served as another critical contributing factor of PMR induced cholestasis. Our present study demonstrated that all three AQs were likely to impair BA homeostasis through direct inhibition of BA transporters as well as altered expression of BA transporters, which probably contributed to PMR induced cholestatic liver injury.