DOCTORAL THESIS

Application of liquid chromatography/electrospray ionization mass spectrometry for bio-analysis and for drug metabolism and pharmacokinetic study of ginsenosides from ginseng

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Application of Liquid Chromatography/Electrospray Ionization Mass Spectrometry for Bio-analysis and for Drug Metabolism and Pharmacokinetic Study of Ginsenosides from *Ginseng*

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Abstract

This work focuses on the application of liquid chromatography-electrospray ionization mass spectrometry in bio-analysis and for drug metabolism and pharmacokinetic of ginsenosides from ginseng.

Qualitative and quantitative analyses of AMP, ADP and ATP in cellular samples have been investigated by using the developed ion-pairing liquid chromatography coupled with electro-spray mass spectrometry (LC-ESI-MS) method. The use of N, N-dimethylhexylamine (DMHA) as the ion-pairing agent enabled the retention and separation of these polar compounds on the reversed-phase HPLC column and the detection of their adduct ions with DMHA by using positive ion mode of ESI-MS. Adduct ions of the nucleotide and the ion-pairing agent DMHA were selected as quantitative ions because their peaks were more intensive than those of intact protonated molecular ions. Linear calibration curves were obtained for AMP with a concentration range of 0.1-20 µM ($y=1607x-84$, $R^2=0.9992$), for ADP at 2-20 µM ($y=3174x-5889$, $R^2=0.9994$), and for ATP at 2.5-20 µM ($y=411x-997$, $R^2=0.9991$), respectively, where y is the peak area and x is the concentration (µM) of the nucleotide. The limits of detection were 0.17 ng for AMP, 4.27 ng for ADP and 6.34 ng for ATP per on-column injection with an injection volume of 5µl. The method has been applied to determine AMP, ADP and ATP concentration levels in biological samples of rat brain cells that were pre-treated with different Zn concentrations to support investigation of Zn's effects to the cell energy metabolism.

Liquid chromatography coupled with mass spectrometry (LC-MS) and tandem mass spectrometry (MS-MS) have been applied to investigate pharmacokinetics and metabolism of ginsenoside Rb_1, Rg_3 and Rh_2.

Pharmacokinetic study of Rg_3 on rat showed short half-life (18.5 min). No Rg_3 and its possible metabolites were detected from the rat urine collected from 0 to 24 hours after intravenous and oral administration. Six metabolites of Rg_3 were detected from feces collected between 0-24 hours after oral administration by LC-MS and confirmed by MS-MS analyses. They are monooxygenated Rg_3, dioxygenated Rg_3, Rh_2, protopanaxadiol, monooxygenated protopanaxadiol and dioxygenated protopanaxadiol. Oxygenation and deglycosylation were found to be the major metabolic pathway of Rg_3 in rat gastrointestinal tract. *in vitro* studies showed that Rg_3 was decomposed to the hydrolysis metabolites, Rh_2 and protopanaxadiol when incubated with 0.1 M HCl. When incubated with rat S9 fraction, monooxygenated metabolite of Rg_3 was produced.

Pharmacokinetic study of Rb_1 on rat gave the long half-life (16.7 hrs.). 4.75-4.94% of the dosage (5mg/kg) was detected from urine sample after intravenous administration. However only 0.02-0.37% of the dosing amount of Rb_1 was detected in the urine sample after oral administration. Nine metabolites, monooxygenated Rb_1, dioxygenated Rb_1, ginsenoside Rd, deglycosylated Rb_1, Rg_3 or F_2, Rh_2 or C-K, monooxygenated Rh_2 or C-K, protopanaxadiol and monooxygenated protopanaxadiol, were detected from urine or feces samples after intravenous and oral administration. Oxygenation and hydrolysis were found to be the major metabolic pathway of Rb_1 in rat.

Pharmacokinetics study shows the short half-life (16 min) of Rh_2 on rat. Three metabolites of Rh_2, monooxygenated Rh_2, protopanaxadiol and monooxygenated protopanaxadiol, were detected from feces collected between 0-48 hours after oral administration by LC-MS and confirmed by MS-MS analyses. Oxygenation and deglycosylation were found to be the major metabolic pathway of Rh_2 in rat

gastrointestinal tract. Moreover, no Rh_2 and its possible metabolites were detected from rat urine sample after intravenous and oral administration.

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