

DOCTORAL THESIS

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

Qian, Tianxiu

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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***

QIAN Tianxiu

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Principal Supervisor: Dr. CAI Zongwei

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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 5 μl . 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 Rb₁, Rg₃ and Rh₂.

Pharmacokinetic study of Rg₃ on rat showed short half-life (18.5 min). No Rg₃ and its possible metabolites were detected from the rat urine collected from 0 to 24 hours after intravenous and oral administration. Six metabolites of Rg₃ were detected from feces collected between 0-24 hours after oral administration by LC-MS and confirmed by MS-MS analyses. They are monooxygenated Rg₃, dioxygenated Rg₃, Rh₂, protopanaxadiol, monooxygenated protopanaxadiol and dioxygenated protopanaxadiol. Oxygenation and deglycosylation were found to be the major metabolic pathway of Rg₃ in rat gastrointestinal tract. *in vitro* studies showed that Rg₃ was decomposed to the hydrolysis metabolites, Rh₂ and protopanaxadiol when incubated with 0.1 M HCl. When incubated with rat S9 fraction, monooxygenated metabolite of Rg₃ was produced.

Pharmacokinetic study of Rb₁ 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 Rb₁ was detected in the urine sample after oral administration. Nine metabolites, monooxygenated Rb₁, dioxygenated Rb₁, ginsenoside Rd, deglycosylated Rb₁, Rg₃ or F₂, Rh₂ or C-K, monooxygenated Rh₂ 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 Rb₁ in rat.

Pharmacokinetics study shows the short half-life (16 min) of Rh₂ on rat. Three metabolites of Rh₂, monooxygenated Rh₂, 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 Rh₂ in rat

gastrointestinal tract. Moreover, no Rh₂ and its possible metabolites were detected from rat urine sample after intravenous and oral administration.

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