DOCTORAL THESIS

Development and application of liquid chromatography and electrospray-ionization mass spectrometry methods for herbal medicine analysis and for the studies of metabolism, DNA adducts and metabonomics of aristolochic acids

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Development and Application of Liquid Chromatography and Electrospray-Ionization Mass Spectrometry Methods for Herbal Medicine Analysis and for the Studies of Metabolism, DNA Adducts and Metabonomics of Aristolochic Acids

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Abstract

Aristolochic acid (AA) is a mixture of structurally related nitrophenanthrene carboxylic acids derived from herbal species *Aristolochia* and *Asarum*. Major components of AA include AAI and AAII. This thesis focuses on the application of liquid chromatography and mass spectrometry for the herbal medicine analysis and for the studies of metabolism, DNA adducts and metabonomics of nephrotoxic and carcinogenic AA.

For the high nephrotoxicity and carcinogenicity of AA, identification of AA containing herbs is important. We described in the present thesis, a new, sensitive and selective HPLC method with fluorescence detector (HPLC-FLD) for the determination of AA in herbal medicines by using pre-column derivatization with zinc powder in acetic acid. The detection limits of developed method were 2-4 orders of magnitude lower than those from existing methods. The HPLC-FLD method has been applied for the determination of AA in herbal medicines.

Drug metabolism study is important for understanding the pharmaceutical or toxicological effect of a drug. In this study, *in vitro* and *in vivo* metabolism studies were conducted so as to gain knowledge about the metabolism and metabolic activation of AA. *In vitro* study was performed by the incubation of AA with rat liver S9, under aerobic or anaerobic conditions. The *in vivo* phase I and phase II metabolism of AA were studied by using Sprague-Dawley rats as the animal model. Structure elucidation of the metabolites was performed by using liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

The formation of DNA adducts is the first step of chemical carcinogenesis. In this study, the feasibility of DNA aristolochic acid (DNA-AA) adducts analysis by

LC-ESI-MS was explored. When DNA was incubated with AA *in vitro*, dG-AAI, dG-AAII, dA-AAI, dA-AAII, dC-AAI and dC-AAII were detected and characterized. The developed LC-MS method was also applied to the identification and quantification of DNA-AA adducts in the kidney and liver DNA samples from the AA dosed rats. dA-AAII was found to be the most abundant among the detected DNA-AA adducts, such as dA-AAII, dA-AAI and dC-AAII. The dC-AA adducts were identified in the *in vitro* and *in vivo* systems, for the first time.

DNA binding assays demonstrated that carcinogens show site- and sequence-specificity and the biological consequence is defined by the nature of binding as well as their position in the genome. In this study, ESI-MS/MS was applied for the identification and position mapping of the DNA-AA adducts in oligonucleotides (ONDs). The observation of the modified bases (modified adenine and guanine) together with the complementary product ions ($[a_n\text{-}B^*_n]^-$, w^-) from the cleavage of the 3' C-O bond adjacent to the modified base in MS/MS analyses readily enabled identification of the AA binding site in ONDs.

The toxic effects of AA to the rats were also investigated by LC-ESI-MS using the metabonomics approach. Analysis of the urine and plasma samples revealed distinct changes in the biochemical patterns in the AA dosed rats. After peak finding and alignment, principal component analysis and partial least squares discriminant analysis were used for multivariate data analysis. Potential biomarkers were studied by high-resolution MS and MS/MS analyses. Citric acid and a glucuronide-containing metabolite were observed as potential biomarkers in rat urine. A significant increase in plasma creatinine concentration was also observed in the AA dosed rats, which indicated that AA induced an adverse effect on the renal clearance function of the animals.

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