Abstract

Study of Intestinal Bacterial Metabolism and Absorption of the Main Ginsenosides in *Panax notoginseng* on *in vitro* models

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Ginsenosides are the major bioactive components in the species of *Panax*, including *P. ginseng, P. quinquefolium and P. notoginseng.* The major ginsenosides in those herbal medicines belong to dammarane-type triterpene O-glycosides: protopanaxadiol (PDS) and protopanaxatriol (PTS) type ginsenosides. Ginsenoside Rb1 and Rg1 are among the most abundant PDS and PTS type ginsenosides, respectively. Various pharmacological activities of Rb1 and Rg1 have been demonstrated in both *in vivo* and *in vitro* models. Therefore, Rb1 and Rg1 are considered as the main bioactive components of these Panax species. However, the oral bioavailability of ginsenosides Rb1 and Rg1 are very low. Intestinal bacterial metabolism is believed to play an important role in production of various metabolites via stepwise hydrolysis. So far, there are many reports on the biotransformation of ginsenosides, especially Rb1 and Rg1 by intestinal bacteria. However, information on some aspects of Rb1 metabolic pathway is still lack; Time-courses of Rb1, Rg1 biotransformation and the main metabolites generation, which will provide important information on the roles of metabolites in actions of ginsenosides, have not yet been reported. Furthermore, whether Rb1 and Rg1 can be suitable markers for prediction of the *in vivo* fates of the PDS- and PTS-type of ginsenosides has not been clarified. In addition, whether the main metabolites formed via hydrolysis by intestinal bacteria bears better permeability, which enable them the main components contributing to the therapeutic outcomes of ginsenosides, are still unclear. The present study aims to provide necessary information for the above questions through biotransformation and absorption studies on *in vitro* human intestinal bacteria model and Caco-2 cell model.

First, a rapid and easily operated *in vitro* model was developed to study biotransformation of ginsenosides by human intestinal bacteria after oral intake. The model was optimized and validated by comparing Rb1 metabolism by human

intestinal bacteria using different medium, incubation mode, analytical conditions etc

Second, Rb1 biotransformation by the human intestinal microflora was studied. Rb1 was incubated with microflora samples from 58 individual healthy volunteers and incubates collected at 12 hr and 18 hr were compared by using HPLC-UV and metabolites identified by using HPLC-MS analyzes. The results showed the major metabolic pathway of Rb1 was Rb1→Rd→F2→Cpd K, and demonstrated, for the first time, the existence of a minor metabolic pathway: Rb1→G-XVII→F2/G-LXXV →Cpd K. Data also exhibited great interindividual variation of Rb1 biotransformation and formation of each metabolite. There were no gender and age difference in this interindividual variation.

Then, the metabolic profiles of Rb1 and Rg1 by pooled human intestinal bacteria were compared with those of PDS (contains equal amount of Rb1) and PTS (contains equal amount of Rg1) isolated from *P. notoginseng*, respectively. The results showed that both Rb1 and Rg1 were cleared rapidly from the incubation system. Rd and compound K, and PPD were the major metabolite during the early (within first 4 hrs), medium (8hrs) and later (after 48hrs) stages of incubation of Rb1, respectively. PPT is the main metabolite of Rg1 and reached its peak level after 15 hrs incubation. The formation of the end product PPD from Rb1 is slower than PPT from Rg1. When PDS and PTS were incubated with human intestinal bacteria, their metabolic patterns were similar to those of Rb1 and Rg1, respectively, when compared the rates of disappearance of Rb1 and Rg1, the time course and levels of the main metabolites formation. The results suggested that Rb1 and Rg1 can reflect the metabolic patterns of PDS and PTS in *P. notoginseng*. Minor interactions of Rb1 and Rg1 with other ginsenosides in PDS and PTS, respectively, were also revealed.

Finally, the permeability of Rb1 and its major metabolites Rd, F2 and Cpd K were compared on Caco-2 monolayer model to find out whether the intestinal bacterial biotransformation produces metabolites bearing better absorption properties. Limited by the availability of analytical instrument, chemphysico-properties of tested ginsenosides and the maximum concentrations can be utilized on basis of MTT cytotoxicity assay, Papp values were not obtained for comparison. But the preliminary data calculated with limited permeated amounts of each compound

indicated better permeability of Rb1 metabolites in ascending order with their formation.

In conclusion, metabolic profiles of main ginsenosides Rb1, Rg1 were elucidated and compared with those of the PDS and PTS from *P. notoginseng* on a human intestinal bacterial *in vitro* model. Results indicated Rb1 and Rg1 as good markers for *in vivo* fates of PDS and PTS. Preliminary data on permeability of Rb1 and its main metabolites on Caco-2 model indicated an important role of Rb1 metabolites in actions of Rb1 and PDS.

Keywords: *Panax notoginseng*; Ginsenoside Rb1; Ginsenoside Rg1; Human intestinal microflora metabolism; Caco-2 cell model