DEGRADATION OF GINSENOSES IN HUMANS AFTER ORAL ADMINISTRATION

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ABSTRACT:

Even though the degradation of ginsenosides has been thoroughly studied in animals and in vitro using acids, enzymes, and intestinal bacteria, knowledge concerning the systemic availability of ginsenosides and their degradation products in humans is generally lacking. Therefore, the attention in this article is focused on the identification of ginsenosides and their hydrolysis products reaching the systemic circulation in man. This is of great importance in understanding clinical effects, preventing herb-drug interactions, and optimizing the biopharmaceutical properties of ginseng preparations. Using a sensitive mass spectrometric method, which is specific for the identification of ginsenosides in complex biological matrices, the degradation pathway of ginsenosides in the gastrointestinal tract of humans could be elucidated following the oral administration of ginseng. Within the frame of a pilot study, human plasma and urine samples of two subjects were screened for ginsenosides and their possible degradation products. In general, the urine data coincided well with the plasma data. In both volunteers the same hydrolysis products, which are not originally present in the Ginsana extract (Pharmaton S.A., Lugano, Switzerland) ingested, were identified in plasma and urine. It was shown that two hydrolysis products of the protopanaxatriol ginsenosides, namely G-Rh1 and G-F1, may reach the systemic circulation. In addition, compound-K, the main intestinal bacterial metabolite of the protopanaxadiol ginsenosides, was detected in plasma and urine. These products are probably responsible for the action of ginseng in humans. In opposition to previous reports, G-Rb1 was identified in plasma and urine of one subject.

Herbal medicinal products are increasingly gaining popularity all over the world. With annual sales of more than $300 million accounting for a 15 to 20% market share in the United States (Gillis, 1997), ginseng is one of the most commonly used herbal medicinal remedies by American consumers (Eliason et al., 1997; Harnack et al., 2001). Also in Europe, ginseng is counted among the top retail products sold in 1996 (Gruenwald and Buettel, 1996), but unlike in the United States, where ginseng preparations are classified as dietary supplements, ginseng products in Europe and particularly in Germany are treated as drugs.

Ginseng, mainly used as a general tonic (Attele et al., 1999), refers to the genus Panax, which includes Panax ginseng C.A. Meyer (Korean ginseng) and Panax quinquefolius L. (American ginseng) (Soldati, 2000). Pharmacological properties of ginseng are generally attributed to triterpene β-glycosides, called ginsenosides. Depending on their aglycone, they are either derivatives of the protopanaxadiol or the protopanaxatriol group (Fig. 1).

In a recent article, Chang et al. (2002) studied the effect of standardized Panax ginseng extract (G115), standardized P. quinquefolius extract (NAGE) and individual ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1) on cytochrome P450 catalytic activity in vitro. The findings indicate that standardized G115 and NAGE extracts decreased human recombinant CYP1A1, CYP1A2, and CYP1B1 activities in an enzyme-selective and extract-specific manner, whereas the isolated ginsenosides, either individually or as a mixture, did not influence the catalytic activity except at higher concentrations. Unfortunately, the effect of the degradation products of ginsenosides on cytochrome P450 catalytic activity was not examined.

Many experiments on the degradation of ginsenosides in the gastrointestinal tract were undertaken using acids, enzymes, intestinal bacteria, and animals (Odani et al., 1983; Strömblom et al., 1985; Karikura et al., 1990; Hasegawa et al., 1996; Akao et al., 1998). From these experiments it is known that protopanaxatriol ginsenosides are hydrolyzed to ginsenoside Rh1 and its hydrated form under mild acidic conditions similar to gastric fluid (Han et al., 1982). Protopanaxadiol ginsenosides are mainly converted to compound-K (C-K1) by intestinal bacteria via stepwise cleavage of the sugar moieties (Hasegawa et al., 1996). Although the degradation pathway of ginsenosides in animals has been well examined in vivo and in vitro, only very few studies have been performed in humans.

Cui et al. (1997) determined the total amount of protopanaxatriol and protopanaxadiol ginsenosides as aglycones in human urine. The results showed that about 1.2% of the orally ingested dose of protopanaxatriol ginsenosides (3 mg) and considerably smaller amounts of the protopanaxadiol ginsenosides not exceeding 0.2% of the administered dose (7 mg) were recovered. However, neither the individual ginsenosides nor their metabolites could be identified. Compound-K, the main intestinal bacterial metabolite of protopanaxadiol ginsenosides, was identified in human serum by a specific enzyme immunoassay 8 h after the oral administration of ginseng (Shibata, 2001).

Nevertheless, it remains to be clarified whether intact ginsenosides
can be absorbed at all from the human gastrointestinal tract and which hydrolysis products of the protopanaxadiol and protopanaxatriol group reach the systemic circulation. Therefore, attention was focused in this study on screening human plasma and urine for the presence of any ginsenosides and their possible degradation products after the oral administration of Ginsana (Pharmaton S.A., Lugano, Switzerland), a commercial ginseng preparation. For this purpose, a sensitive mass spectrometric method that is specific for the identification of ginsenosides in complex biological matrices has been developed (manuscript in preparation).

![Chemical structures and m/z values of ginsenosides and their main degradation products in addition to their major fragment ions observed in the first fragmentation step (MS²).](image)

<table>
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<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>m/z</th>
<th>m/z of major fragments</th>
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<td></td>
<td></td>
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<tr>
<td>Ginsenoside G-Rb₁</td>
<td>-O-Glc²⁻¹Glc</td>
<td>-H</td>
<td>-O-Glc⁶⁻¹Glc</td>
<td>1131</td>
<td>789, 365</td>
</tr>
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<td>G-Rb₂</td>
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<td>-H</td>
<td>-O-Glc⁶⁻¹Arap</td>
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<td>789, 335</td>
</tr>
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<td>G-Rc</td>
<td>-O-Glc²⁻¹Glc</td>
<td>-H</td>
<td>-O-Glc⁶⁻¹Araf</td>
<td>1101</td>
<td>789, 335</td>
</tr>
<tr>
<td>G-Rd</td>
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<td>-H</td>
<td>-O-Glc</td>
<td>969</td>
<td>789</td>
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<td>-H</td>
<td>-OH</td>
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<tr>
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<td>-H</td>
<td>-O-Glc</td>
<td>645</td>
<td>203</td>
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<td><strong>Protopanaxatriol-type</strong></td>
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<td>-O-Glc</td>
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<tr>
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<td>-O-Glc²⁻¹Rha</td>
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<td>203</td>
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<tr>
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<td>-OH</td>
<td>-OH</td>
<td>-OH</td>
<td>477</td>
<td>459, 441, 423</td>
</tr>
</tbody>
</table>

Glc, β-D-glucopyranosyl; Arap, α-L-arabinopyranosyl; Arafp, α-L-arabinofuranosyl; Rha, α-L-rhamnopyranosyl. * the degradation products of ginsenosides detected in this study.

FIG. 1
Materials and Methods

**Chemicals and Reagents.** Purified individual ginsenosides Rb₁ and Rg₁ were purchased from Extrasynthese (Genay, France). Rb₂ from CANPO Chemicals Co., Ltd. (Chengdu, China), and panaxatriol from Sequoia Research Products Ltd. (Oxford, UK). Other ginsenosides were kindly supplied by Indena (Milano, Italy). Human plasma used for preliminary tests was generously donated by AAI Deutschland (Neu-Ulm, Germany). Ginsana G115 capsules from Pharmaton S.A. (Lugano, Switzerland) were used for the pilot study.

Methanol and acetonitrile, both of high-performance liquid chromatography grade, were obtained from Carl Roth (Karlsruhe, Germany). Perchloric acid 70% analytical grade was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Water was purified by a Milli-Q system (Millipore, Bedford, MA) and used for all aqueous procedures.

**Pilot Study.** The following pilot study was carried out in accordance with German law concerning the performance of clinical studies. Based on the German Commission E daily recommendation of 1 to 2 g of ginseng root, seven capsules of Ginsana each containing 100 mg of G115 standardized to 4% ginsenosides were taken orally as a single dose on an empty stomach by two healthy volunteers. Predose blood and urine samples were taken 30 min before drug administration.

Overall, 25 blood samples were obtained from each subject. During the first 2 h and between 7 and 12 h after drug intake, blood samples were collected every half hour. In the time between 3 and 7 h as well as 12 and 15 h after drug administration, blood was taken every hour. Starting from 15 until 24 h after drug administration, blood was obtained every 3 h. With each sampling 10 ml of blood was collected in a K3E Vacutainer (Becton Dickinson, Heidelberg, Germany) and centrifuged immediately to yield plasma.

Urine samples were collected in four fractions: 0 to 3, 3 to 6, 6 to 12, and 12 to 24 h after the oral administration of Ginsana. All plasma and urine samples were stored at −20°C until time of analysis.

**Sample Preparation from Plasma.** Volumes of 1 ml of plasma were acidified with 12.5 μl of perchloric acid (35%) and kept on ice for 30 min. Afterward, they were centrifuged for 3 min at 1100g in a Varifuge 3.0 R (Heraeus Sepatech, Osterode, Germany) to discard the precipitated protein. Then the supernatant was subjected to solid-phase extraction using Bond-Elut C₁₈ 200-mg cartridges from Varian, Inc. (Palo Alto, CA) after equilibrating with 3 ml of methanol and 3 ml of water. Under gentle vacuum the liquid was passed through the cartridge, which was then washed five times with water and dried thereafter. Finally, the glucosylated ginsenosides were eluted twice with 250 μl of an acetonitrile/water mixture (1:1), followed by the elution of the aglycones with 2 × 250 μl of methanol. These eluents were then subjected to mass spectrometric analysis using electrospray ionization (ESI).

**Sample Preparation from Urine.** One milliliter of urine was extracted with 1 ml of butanol. After washing three times with 1 ml of water, the organic phase was evaporated under a stream of argon. The residue was dissolved in water and subjected to solid-phase extraction following the procedure described above.

**Sample Preparation of Ginsana Capsules.** Three Ginsana capsules were extracted three times with 20 ml of 20% aqueous methanolic solution for 30 min at 55°C, respectively. The combined extracts were evaporated to dryness under reduced pressure at 40–55°C. At last, the residue was dissolved in water and applied to Bond-Elut C₁₈ 200-mg cartridges as described above.

**Instrumentation.** All ESI-MS and ESI-MSⁿ measurements were performed with an LCQ quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with a Nano-ESI-source (Protana, Odense, Denmark). Laboratory gold-coated glass capillaries were filled with 3 μl of sample solution and used for electrospray by application of a voltage of 1000 V. The transfer capillary was held at 200°C. The capillary and the tube lens were held at the same potential (48 V). For fragmentation, the relative collision energy in the trap, depending on the structure of the precursor ion, was set between 30 and 70% (corresponding to the LCQ software settings and defining the amplitude of the resonance excitation a.c. voltage). All positively charged ions produced of the ginsenosides appeared as [M + Na]^+ ions.

**Results**

Before studying the degradation of ginsenosides in humans, the ginsenosides present in Ginsana G115 capsules were identified by ESI-MS. The corresponding mass spectrum in Fig. 2 shows all ginsenosides detected being G-Rg₁, G-Rb₁, G-Rb₂/G-Rc, G-Rd, G-Re, and G-Rf. As known from previous analyses, G-Rb₁, G-Rb₂/G-Rc, G-Rd, G-Re, and G-Rf represent quantitatively the main components of the protopanaxadiol ginsenosides in Ginsana G115 capsules, whereas G-Rg₁ and G-Re are the main components of the protopanaxatriol ginsenosides (Chang et al., 2002).

Although having the same mass, the presence of G-Rd/G-Re and G-Rg₁/G-Rf in the standardized G115 extract could be individually
proven by means of consecutive fragmentation steps (spectra not shown). Only G-Rb₂ and G-Rc differing just in the ring size of the arabinose sugar were not distinguished.

The detection of ginsenosides and their corresponding degradation products in human plasma and urine was based on the identification of their characteristic fragment ions in the tandem mass spectrometry mode, because at concentrations lower than 100 ng/ml, the signals of the ginsenosides can no longer be discerned from the noise in the full-scan spectrum. Based on previous experiments (Abdel Tawab et al., 2000), the fragmentation pattern of all ginsenosides, their possible degradation products, and their aglycones were established (see Fig. 1). The detection limit was approximated as the concentration that gives a signal-to-noise ratio of 2 within a mass window of 15 Da centered on the exact mass of the fragment ion. Depending on their ionization efficiency, the detection limit of ginsenosides in plasma was 8 ng/ml for G-Rg₁, G-Rd, and G-Re; 20 ng/ml for G-Rh₁, G-Rc, and G-Rb₂; and 40 ng/ml for G-Rb₁ and panaxatriol. In urine the limit of detection was 2 ng/ml for G-Rg₁, G-Rd, and G-Re; 5 ng/ml for G-Rb₁, G-Rb₂, and G-Rc; 20 ng/ml for G-Rh₁; and 40 ng/ml for panaxatriol. Blank plasma and urine samples were tested for the absence of these characteristic fragment ions.

Finally, human plasma and urine samples were screened for all detected ginsenosides in Ginsana and their possible degradation products. The results of these experiments are presented in Fig. 3. In general, the urine data coincided well with the plasma data. In both volunteers the same hydrolysis products, which are not originally present in the Ginsana extract, were identified in plasma and urine. Thus, these detected products must result either from the degradation or metabolism of ginsenosides in the human body. Whereas the monoglucosylated ginsenoside detected within 3 h after drug administration can be clearly attributed to G-Rh₁ (see Discussion) the monoglucosylated ginsenoside identified after 8 h may refer to G-Rh₁ or G-F₁. As both hydrolysis products show the same fragmentation behavior, they cannot be differentiated mass-spectrometrically. Further attempts are under way to distinguish between these two monoglucosylated ginsenosides in very low concentration ranges. With regard to the protopanaxadiol ginsenosides, C-K and its hydrated form revealed to be the only hydrolysis products detectable 7 to 8 h after the intake of Ginsana.

The only difference between both volunteers was observed in the detection of G-Rb₁, which was absorbed in its intact form in addition to the previously mentioned hydrolysis products by only one subject. The corresponding spectrum is shown as a representative example in Fig. 4a. In the urine of the same subject, the intact ginsenosides G-Rg₁, G-Rd, G-Re, G-Rb₂, and G-Rc were detected. Figure 4b shows the identification of C-K, the main intestinal bacterial metabolite of the protopanaxadiol ginsenosides, in urine.

**Discussion**

From many other studies on the degradation and metabolism of β-glycosides of plant origin, it is known that the metabolism proceeds mainly via degradation processes already occurring in the gastrointestinal tract (Hasegawa et al., 1996). Thus, the degradation products identified in plasma and urine most probably result from the breakdown of ginsenosides in the gastrointestinal tract caused either by gut microorganisms, intestinal enzymes, or gastric fluid.

Referring to previous experiments on the hydrolysis of protopanaxatriol ginsenosides under acidic conditions, the monoglucosylated protopanaxatriol ginsenoside and its hydrated form detected in the first hours after drug administration can be assigned to G-Rh₁ and hydrated G-Rh₁. They represent the main hydrolysis products of G-Rg₁, which were also obtained by using 0.1 N HCl at 37°C, the acidity of which is similar to gastric fluid (Han et al., 1982). Therefore, the rapid absorption of G-Rh₁ and hydrated G-Rh₁ from the
upper part of the gastrointestinal tract is an indication for the hydrolysis of G-Rg₁ occurring in the stomach.

In contrast, the hydrolysis products of G-Re, namely G-Rg₂ and its hydrated derivative, were not detected in plasma and urine. Except for the presence of a terminal rhamnose at the C₆-sugar moiety, G-Re is very closely related chemically to G-Rg₁. Since only the C₂₀-sugar is eliminated under acidic conditions, it can be assumed that G-Re, being a rhamnosyl derivative of G-Rg₁, is hydrolyzed in a similar manner as G-Rg₁. However, the equivalent hydrolysis products of G-Re could not be detected, probably because they are not absorbed due to the presence of the terminal rhamnose. The inhibitive effect of a terminal rhamnose on the absorption of glycosides has been observed before in

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**Fig. 4. Identification of G-Rb₁ in subject’s plasma on the basis of its specific fragment ions at m/z 789 and m/z 365 (a); and identification of C-K in subject’s urine by means of its characteristic fragment ion at m/z 203 (b).**
the case of quercetin-4-O-rhamnoglucoside. Compared with the glucoside, the rhamnoside derivative was not absorbed from the gastrointestinal tract in humans except after the hydrolysis by microflora later in the colon (Scalbert and Williamson, 2000). Even then, the small amount of the degradation products found reflected a much smaller uptake of the rhamnoglucoside compared with the glucoside (Hollman et al., 1999).

Surprisingly, a monoglucosylated protopanaxatriol (G-Rh1/G-F1) appeared again in plasma 8 h after drug administration. This may be ascribed to the following degradation pathways (Fig. 5).

**Pathway a.** Not all G-Rg1 is hydrolyzed in the stomach. The unchanged part reaches the large intestine, where it is hydrolyzed by intestinal bacteria to G-F1 in the same manner observed by the in vitro incubation with fecal cultures (Hasegawa et al., 1996).

**Pathway b.** The monoglucosylated protopanaxatriol is an intestinal metabolite of G-Re, which is first hydrolyzed in the stomach to yield G-Rg2 and is then converted to G-Rh1 by the elimination of rhamnose through intestinal bacteria.

**Pathway c.** Unhydrolyzed G-Re reaches the large intestine, where it is metabolized by intestinal bacteria to G-F1 via G-Rg1.

Accordingly, the monoglucosylated protopanaxatriol ginsenoside (G-Rh1/G-F1) may represent either an intestinal metabolite of G-Rg1, or G-Re. This preliminary finding is the first indication for an intestinal degradation of protopanaxatriol ginsenosides taking place in humans. It is worth mentioning that mainly the monoglucosylated degradation products of the protopanaxatriol ginsenosides were absorbed and not the corresponding aglycones. This may be attributed to the greater water solubility of monoglucosylated ginsenosides and their enhanced absorption by the intestinal sodium-dependent glucose transporter (SGLT1) (Wolffram et al., 2002).

No degradation products of the protopanaxadiol ginsenosides were detected in plasma and urine in the early hours after drug adminis-
tration, suggesting that protopanaxadiol ginsenosides are hardly decomposed in the stomach. The prolonged time needed for the appearance of C-K and its hydrated form in plasma indicates that the absorption takes place in the lower part of the intestine.

Previous in vitro experiments showed that bacterial intestinal degradation of protopanaxadiol ginsenosides proceeds stepwise via cleavage of the sugar moieties, liberating mainly the monoglucosylated ginsenoside compound-K (Karikura et al., 1990; Hasegawa et al., 1996; Bae et al., 2002). This could be verified by our study. The observed intestinal degradation product of the protopanaxadiol ginsenosides suggests the presence of bacterial β-glucosidase enzymes, that hydrolyze the glycosidic linkage. Human intestinal bacteria having β-glucosidase activity that metabolizes protopanaxadiol ginsenosides to C-K are mainly Enterobacter sp. (Hasegawa et al., 1997), Prevotella oris (Hasegawa et al., 1997), Streptococcus sp., and Biﬁdobacterium sp. (Bae et al., 2000).

Contrary to previous reports (Kato et al., 1990; Akao et al., 1998), intact undecomposed G-Rb1 could be absorbed and remained detectable in plasma for 3 h prior to its excretion after 6 h.

The detection of the other ginsenosides G-Rg1, G-Rd, G-Re, G-Rb2, and G-Rc in urine but not in plasma may be ascribed to the lower limit of detection observed in urine. Based on this observation, the concentration of these intact ginsenosides must be very low, ranging between 2 and 8 ng/ml for G-Rg1, G-Rd, G-Re and 5 to 20 ng/ml for G-Rb2 and G-Rc.

Bearing in mind the very low concentrations of protopanaxatriol and protopanaxadiol ginsenosides recovered in urine previously (Cui et al., 1997), the main aim of this study was to identify the ginsenosides and their degradation products actually reaching the systemic circulation. In summary, it was proven by the above results that two degradation products of the protopanaxatriol ginsenosides, namely G-Rh1 and G-F1, may reach the systemic circulation in humans in addition to compound-K resulting from the stepwise deglycosylation of protopanaxadiol ginsenosides. Furthermore, G-Rh1 could be clearly identified in plasma and urine, in contrast to previous reports.

It seems that the metabolism of the ginsenosides mainly takes place in the gastrointestinal tract, but liver enzymes may also play a role. Thus, further experiments will be conducted in this regard. However, the present identification of the degradation products actually reaching the systemic circulation facilitates further quantification studies and helps to better understand the molecular mechanism of action and the clinical effects of ginseng in the future.

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