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Short communication

Excretion profile of glycyrrhizin metabolite in human urine

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ABSTRACT

Glycyrrhizin is the main pharmacologically active component in licorice. Long term consumption of higher amounts of glycyrrhizin results in serious side effects (pseudoaldosteronism) caused by the glycyrrhizin metabolite 3β-monoglucuronyl-18β-glycyrrhetinic acid (3-MGA). The aim of this study was to determine urine excretion profile of 3-MGA. Six healthy volunteers ingested 600 mg of glycyrrhizin. In the first experimental period, glycyrrhizin was ingested in the morning and in the second experimental period, which followed after 2 weeks, glycyrrhizin was ingested in the evening. Results showed that the amount of excreted 18-MGA in urine was 1425.9–3147.8 μg, which corresponds to 0.31–0.67% of the ingested dose. Maximum elimination rates were 31.52–209.47 μg/h. There were no significant differences in the amount of excreted 3-MGA as well as in elimination rates of 3-MGA when glycyrrhizin was ingested in the morning or evening, respectively. These results provide some important clinical data on glycyrrhizin metabolism.

1. Introduction

Licorice and licorice containing products are very popular in many European countries, especially in the Nordic countries, United Kingdom, Netherlands, and Germany. Few examples of medicinal uses include herbal teas and herbal preparations consumed to improve overall health or alleviate wide range of diseases such as respiratory, gastrointestinal, cardiovascular, urogenital, skin, or eye diseases (Fiore, Eisenhut, Ragazzi, Zanchin, & Armanini, 2005). In contrast, pure glycyrrhizin, the main pharmacologically active component in licorice, also known for its pleasant aromatic sweet taste, is commonly used as a flavouring and sweetening agent in confectionery, beverages, chewing gums, and tobacco products (Carmines, Lemus, & Gaworski, 2005; Gabriele, Curcio, & Cindio, 2001). The European Scientific Committee on Food of the European Commission has estimated an average yearly consumption per person of 1–2.5 kg of licorice containing confectionery for people from the Nordic countries, whereas the average daily consumption among 3% of regular consumers in the Dutch population amounted even more than 50 g, which is 22 kg per year (Scientific Committee on Food, European Commission, 2003). The maximum recommended amount of glycyrrhizin consumption has not been determined precisely, but the European Scientific Committee on Food considers the upper limit for regular ingestion 100 mg/day (Scientific Committee on Food, European Commission, 2003). This amount corresponds to approximately 0.6 g of hard candies, considering that the glycyrrhizin content is at the upper allowed limit of 16% (The Code of Federal Regulations, US Food and Drug Administration, 2010), or to approximately 50 g of common liquorice sweets, considering that the glycyrrhizin content is 0.2% (Størmer, Reistad, & Alexander, 1993). Commission Directive 2004/77/EC requires labelling of products, which give consumers clear information on the presence of glycyrrhizin or its ammonium salt in confectionery or beverages, whereas the US Food and Drug Administration allows its use in food only within the specified limitations (Scientific Committee on Food, European Commission, 2003; The Code of Federal Regulations, US Food and Drug Administration, 2010).

Long term consumption of higher amounts of glycyrrhizin results in serious side effects known as the syndrome of pseudoaldosteronism: sodium, chloride, and water retention, hypokalemia, hypertension, and muscle contraction disturbances (Bruneton, 1999). Pseudoaldosteronism is caused by glycyrrhizin metabolite 3β-monoglucuronyl-18β-glycyrrhetinic acid (3-MGA), which inhibits 11β-hydroxysteroid dehydrogenase, a key enzyme involved in cortisol conversion to cortisone (Kato, Kanaoka, Yano, & Kobayashi, 1995). Since cortisol binds to the aldosterone receptors with the same affinity as aldosterone, the result is a mineralocorticoid effect. Several cases of licorice intoxication have been reported in literature (Guillaume, van der Molen, Kerstens, Dullaart, & Wolthers, 1999; Kato et al., 1995; Kerstens, Guillaume, Wolthers, & Dullaart, 1999) and it has to be emphasised that it is often extremely difficult to establish licorice abuse as the cause of mineralocorticoid symptoms. Therefore, there is an urgent need for clinically applicable diagnostic methods as well as a need for detailed knowledge of glycyrrhizin metabolic behaviour.

After oral ingestion, glycyrrhizin is first hydrolysed to 18β-glycyrrhetinic acid (18-GA) by intestinal bacteria, and after complete

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Table 1
Excretion data of 3-MGA in urine after single oral ingestion of 600 mg of glycyrrhizin (corresponding to 471.6 mg of 3-MGA) by six volunteers.

Volunteer	Time of glycyrrhizin ingestion	Time to first detection [h]	t_{max} [h]	c_{max} [μg/ml]	Maximum elimination rate [μg/h]	Time to maximum elimination rate [h]	Average daily urine volume [ml]	Total amount of excreted 3-MGA [μg]	% of metabolite excreted in urine
1	Morning	7.7	29.5	1.62	117.23	25.9	1071	2455.4	0.52
	Evening	6.5	25.0	2.69	120.83	25.0	1236	3109.8	0.66
2	Morning	3.7	22.2	1.81	58.63	22.1	1083	1425.9	0.31
3	Morning	8.5	25.7	1.41	80.96	54.7	1170	2054.3	0.44
	Evening	14.0	39.5	0.99	122.37	39.6	1580	2811.6	0.61
4	Morning	13.0	28.7	1.05	72.07	28.8	1420	1726.1	0.37
	Evening	10.5	27.5	0.49	99.06	19.2	2560	1490.6	0.32
5	Morning	1.5	1.5	1.15	209.47	1.4	1087	2014.1	0.44
	Evening	3.5	21.5	0.67	31.52	28.1	875	1949.9	0.42
6	Morning	1.8	22.0	2.11	106.31	22.1	1307	2246	0.49
	Evening	12.5	19.5	1.64	96.72	88.1	892	3147.8	0.67

absorption from gut (Takeda et al., 1996), 18-GA is metabolised to 3-MGA in the liver (Ohtake et al., 2007). 3-MGA circulates in the bloodstream (Krähenbühl et al., 1994). The main part is eliminated by bile and only a minor part by urine (Kerstens et al., 1999). Therefore, analytical methods have to be sensitive enough to detect the metabolite at low concentrations. Several HPLC methods for determination of 18-GA in urine, after enzymatic hydrolysis of 3-MGA to 18-GA, have been published (Cantelli-Forti et al., 1994; Hasler, Krapf, Brenneisen, Bourquin, & Krähenbühl, 1993; Kočevar Glavač, Injac, & Kreft, 2010; Krähenbühl, Hasler, & Krapf, 1994; Raggi, Bugamelli, Nobile, Schiavone, & Cantelli-Forti, 1994; Yamamura et al., 1991). However, in contrast to serum pharmacokinetic data, only limited data exist on 3-MGA urine excretion.

The aim of our study was to determine the 18-GA urine excretion profile after oral consumption of water sweetened with glycyrrhizin and to test the usefulness of HPLC method with UV spectrophotometric detection.

2. Experimental

2.1. Chemicals and reagents

18β-Glycyrrhetinic acid (glycyrrhizin) was obtained from Fluka (Seelze, Germany) and β-glucuronidase Type HP-2 from *Helix pomatia* from Sigma (Saint Louis, USA). Acetonitrile (Riedel-de Haën, Seelze, Germany) and methanol (Merck, Darmstadt, Germany) were of chromatographic grade. All other reagents were of analytical grade.

2.2. Study design and collection of urine samples

After giving a written consent to participate in our study, six healthy volunteers (one female and five males aged 24–40 years) ingested 600 mg of glycyrrhizin dissolved in 2 dl of water. The dose was selected according to the German Commission E data on normal therapeutic doses, which range from 200 to 600 mg of glycyrrhizin daily (Blumenthal, 1998). In the first experimental period, glycyrrhizin was ingested in the morning and in the second experimental period, which followed after 2 weeks, glycyrrhizin was ingested in the evening. Volunteers were allowed to eat and drink normally during the phase of collecting urine samples. Every urine excretion was collected separately and the urine volume measured. Urine samples were collected for several days. Blank urine was obtained from the same healthy volunteers prior to ingestion of glycyrrhizin; they did not consume any licorice or glycyrrhizin containing products for at least 3 months. Urine samples were stored in refrigerator for up to 2 days until prepared for analysis.

2.3. Standard solution and urine sample preparation

Stock solution of 18-GA standard was prepared at concentration of 1.0 mg/ml in methanol and stored in refrigerator. The stock solution was diluted with distilled water to obtain concentration 0.1 μg/ml. Urine samples were prepared by first adjusting the urine pH to 5–6 with 1 M HCl, adding the enzyme solution (final concentration 0.5% (v/v), which corresponds to 581 units/ml), and then incubating for 4 h at 37 °C. Prior to HPLC analysis, samples were acidified with 0.1% trifluoroacetic acid (TFA) to pH 1–2 and centrifuged at 10,000 rpm for 3 min.

2.4. Instrumentation and operating conditions

HPLC analyses were performed using Knauer HPLC system: Well Chrom K-2500 detector, Well Chrom K-501 pump, and Knauer degasser (Knauer Wissenschaftliche Gerätebau, Berlin, Germany), equipped with EuroChrom® 2000 Basic Edition software, version 2.05. Chromolith® Performance RP-1 endcapped column, 4.6 × 100 mm (Merck, Darmstadt, Germany) was used. Urine samples were analysed using our previously published method (Kočevar Glavač et al., 2010), which was modified only by 4× increased injection volume. Operating conditions: injection volume 200 μl, flow rate 2 ml/min, detection wavelength 250 nm, mobile phase A distilled water with 0.1% TFA, mobile phase B 100% acetonitrile with 0.1% TFA, elution gradient 0–1 min 100% A, 1–2 min 100–40% A, 2–4 min 40% A, 4–4.5 min 40–30% A, 4.5–6 min 30% A, 6–6.5 min 30–100% A, 6.5–10 min 100% A. The method was previously shown to be specific, accurate, linear ($R = 0.999$), repeatable ($RSD_{AUC} < 3\%$). The limit of detection of this method is 0.01 μg/ml.

2.5. Statistical analysis

Concentration of 18-GA in hydrolysed urine samples was calculated from calibration line obtained by analysis of standard solution. Concentration of 3-MGA in urine samples was calculated by multiplication with factor of molar masses of 3-MGA and 18-GA (646.8:470.7 = 1.374). This concentration was multiplied by volume of urine at each excretion to obtain the amount of excreted 3-MGA. The amount of 3-MGA excreted in each urine excretion was summarised to obtain total amount of excreted 3-MGA, and this was divided by 471.6 mg (which is equimolar to 600 mg of glycyrrhizin) to obtain portion of excreted metabolite. The amount of 3-MGA excreted in each urine excretion was also divided by time interval between two excretions to obtain excretion rate (expressed in μg/h). Pearson correlation test and *t*-test were calculated with SPSS statistical program.