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The ginseng's fireness is associated with the lowering activity of liver Na^+-K^+-ATPase

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ABSTRACT

Ethnopharmacological relevance: Ginseng is an herbal medicine used worldwide that possesses a wide range of pharmacological activities. However, its side effects are rarely discussed. The experience of Chinese medicine has revealed that taking ginseng at a high dose chronically can cause *fireness*, *i.e.*, the ginseng-abuse syndrome. Here, we explored the mechanism of ginseng's *fireness* by comparing the energy metabolism of mice affected by red ginseng (RG), ginseng (GS), ginseng leaves (GL) and American ginseng (AG), which exhibit different drug properties according to the theory of TCM.

Materials and methods: KM mice were randomly divided into five groups ($n \geq 30$ per group) and administered distilled water or drugs, respectively. Mice receiving RG, GS, or GL received 4.5 g/(kg day), while the mice receiving AG received 3 g/(kg day). Control mice received distilled water. The duration of exposure for all groups was 31 days. The mice's physical characteristics, such as eye condition, rectal temperature, saliva secretion, urine, stool weight, blood coagulation time and swimming time, were measured at different times after administration. Energy metabolism indexes were measured via TSE phenoMaster/LabMaster animal monitoring system, including the mice' 24 h oxygen consumption (VO_2), carbon dioxide production (VCO_2), heat production (H) and energy expenditure (EE). Biochemical indices were measured by ultraviolet spectrophotometer and microplate reader, including pyruvic acid content in serum and succinate dehydrogenase (SDH) activity, lactate dehydrogenase (LDH) activity, the Na^+-K^+-ATPase activity and the content of glycogen in the liver tissue.

Results: After 31 days of drug administration, mice in the RG and GS groups exhibited obviously more eye secretions, less saliva secretion and less urine. Compared with the control group, the swimming times of mice in the GS, AG and GL groups were significantly prolonged; the clotting time of mice in the GL was extended significantly; VCO_2, H and EE of mice in the GS group were obviously increased; Pyruvate content of mice in the RG group showed an initial decrease followed by an increase; SDH activity of mice in the AG and GL groups was significantly inhibited; LDH activity of the mice showed no significant difference among different groups; Na^+-K^+-ATP enzyme activity of the RG and GS groups showed up-regulation initially and then down-regulation; the content of hepatic glycogen of mice in the GS and GL groups increased significantly.

Conclusion: The results demonstrated that RG and GS with their warm drug nature could enhance the body's energy metabolism to produce their *dryness* to the body. The liver Na^+-K^+-ATP enzyme activity may be the primary index for indicating the *fireness* of ginseng. In addition, our results demonstrated that ginseng, especially red ginseng, is not suitable for long time application with a higher dose.

1. Introduction

Ginseng, the dried root and rhizome of *Panax ginseng* C. A. Meyer, is a famous drug widely used for thousands of years throughout the world. The Chinese regarded it as a tonic for the restoration of strength, and so a panacea name was given to it (hence, the genus Panax, meaning all healing) (Siegel, 1979). Modern experiments have also indicated that ginseng possesses various pharmacological activities (Takagi et al., 1972). The long-

Abbreviations: AG, American ginseng; GS, ginseng; RG, red ginseng; GL, ginseng leaves; VO_2, oxygen consumption; VCO_2, carbon dioxide production; H, heat production; EE, energy expenditure; SDH, succinate dehydrogenase; LDH, lactate dehydrogenase

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term application of ginseng in Chinese medicine has found that if it is used chronically or in higher doses, it can cause *fireness* (火, in Chinese), i.e ginseng-abused syndrome. Research has indicated that the side effects of palpitations, sleeplessness and dizziness seen with ginseng may be associated with the excitation of the central nervous system, as well as a cardiotonic action (Wu, 1981). Chinese clinical experiments have indicated that patients appeared with symptoms of dizziness, palpitations, blurred vision, dry heat, and body burnout after receiving RG 15 g as a one-time dose (Wu, 1981). In Chinese medicine, the highest dose of ginseng that has been given is up to 50 g. The clinical observation showed that large doses of ginseng could induce insomnia, depression, and nervous disorders (Keys, 1976). To confirm the side-effects of ginseng, a rigid clinical experiment was performed that indicated that 14 of 133 long-term ginseng users (10%) exhibited side-effects. Patients who ingested an average daily dose of 3 g experienced hypertension, nervousness, sleeplessness, skin eruptions and morning diarrhea; some subjects also became euphoric and agitated. Doses of 15 g were associated with depersonalisation and confusion, while depression was reported after more than 15 g of ginseng per day (Siegel, 1979). As usual, the theory of traditional Chinese medicine (TCM) is that the ginseng *fireness*, i.e., ginseng-abuse syndrome, is caused by its *dryness* (燥性 in Chinese), which was caused by its mild property (Su and Sun, 2015). The property theory of TCM mainly includes four *Qi* (in Chinese), five flavors, and channel tropism, etc. The four *Qi* referred to are *cold, cool, mild* and *warm*. Recently, a new theory has been presented that the four *Qi* of Chinese drugs is mostly associated with energy metabolism (Huang et al., 2011). In addition, pharmacological testing has also revealed that the stimulant effects of ginseng, which provide the anti-fatigue and enhanced performance effects, may be associated with the alteration of carbohydrate metabolism and increased synthesis of glycogen and phosphorous compounds induced by ginseng (Brekham and Dardymov, 1969). These studies led us to study the *fireness* of ginseng. According to the Chinese Pharmacopoeia, red ginseng exhibits the warm property, ginseng or dried ginseng shows the mild property, ginseng leaf presents the cold property and American ginseng shows the cool property.

In addition, these four medicinal materials contain similar ginsenosides, but the proportions of main individual ginsenosides between the materials are different. Typically, ginsenosides can be classified into protopanaxadiol (PPD), protopanaxatriol (PPT) and oleanolic acid types according to their aglycone. The PPD and PPT are the predominant ginsenosides in the four drugs. Ginsenoside-Re, derived from the PPT-type, is the highest in ginseng leaves and ginsenoside-Rb_1, derived from PPD-type, is the highest in American ginseng. The total content of ginsenoside-Re and -Rg_1 derived from the PPT-type is almost equal to -Rb_1. The ginseng root contains all of these ginsenosides in higher concentrations. Other ginsenosides, such as ginsenoside-Rh_1, -Rh_2,-Rg_3, -Rg_5 and -F_4, formed either from glycosyl elimination or oxidation in the side-chain of ginsenoside-Rb_1 or –Re during the steaming process for RG by heating, are higher in the RG (Ma et al., 1996). In traditional Chinese medicine, the usage for RG and GS was different. GS was mainly used for its tonic action, such as anti-fatigue, but RG was mainly used for frail or asthenic patients. Present research indicates that RG is stronger than GS in anticancer actions (Zheng et al., 2012). Previously, the characteristic constituents of ginseng and American ginseng, as well as the activities of ginsenosides were engaged, indicating that the individual ginsenosides exhibited different activities (Dou et al., 1998; Xiang et al., 2013a, 2013b; Zhang et al., 2013). Thus, all of the ginsenosides together, in different proportions, could embody different actions. As the primary distinction between the four drugs in TCM is the difference of their properties, the comparison of their effects on energy metabolism could reflect a difference in their properties. In general, the side effects of ginseng that occurred in some individuals included high blood pressure, insomnia, restlessness, anxiety, euphoria, diarrhea, vomiting, headache, nosebleeds, breast pain and vaginal bleeding (Jia et al., 2009).

Generally, the ancients used other drugs along with ginseng to counteract ginseng's *fireness*, called a composite prescription. However, currently the gardened ginseng is only used for food, which has been approved by Chinese government. Thus, the research into ginseng's *fireness* is significant and imperative. However, there is currently very little research on the topic. In this paper, we took GS, RG, GL and AG as examples to study the relationship between each ginseng's *fireness* and its action on energy metabolism to reveal its mechanism of action. First, the appropriate dosages of GS, RG, GL and AG were determined by converting the doses for humans listed in the Chinese Pharmacopoeia to doses for mice based on body surface area (BSA). This paper addresses the time-dependent effects of the four drugs on energy metabolism.

2. Materials and methods

2.1. Materials and reagents

Red ginseng (Batch No. 20131009) was purchased from Dalian, China, in October 2013. Ginseng (Batch No. 20131010) was provided by Benxi Ginseng Cultivation Farm, Liaoning province of China. Ginseng leaves (Batch No. 20141012) were obtained from Kuan Dian, Liaoning province of China and American ginseng (Batch No. 20141015) was purchased from a pharmacy store in Dalian, Liaoning Province of China. All of the samples were identified by Professor Wang Bing (Liaoning University of Traditional Chinese Medicine) as the dried roots and leaves of *Panax ginseng* C. A. Meyer, as well as the dried root of *Panax quinquefolium* L, respectively. All of the voucher specimens were deposited in the herbarium and analysed to meet with the standards of China's Pharmacolopoeia. Assays were performed using the protein quantitative assay kit (Batch No. 20140829, Nanjing Jian Cheng Bioengineering Institute), glycogen assay kit (Batch No.20140901, Nanjing Jian Cheng Bioengineering Institute), SDH kit (Batch No.20140903, Nanjing Jian Cheng Bioengineering Institute), LDH kit (Batch No. 20140829, Nanjing Jian Cheng Bioengineering Institute), pyruvate assay kit (Batch No. 20140903, Nanjing Jian Cheng Bioengineering Institute), and Na^+-K^+-ATPase assay kit (Batch No. 20140903, Nanjing Jian Cheng Bioengineering Institute).

2.2. Sample preparation

The mice were administered crude drug powder because there is no extraction procedure. The procedure for sample preparation was as follows: GS, GL and AG were crude drug samples, but RG is a processed form of GS. The samples of RG, GS, GL and AG were heat-dried at 50 °C, pulverized to pass through a 150-mesh sieve and then the powders were dissolved in distilled water for administration. Owing to the fact that GL is all leaf materials, it is lighter than RG, GS and AG. Additionally, triple the BSA-adjusted reference dose of GL was too thick to conduct intragastric administration. Therefore, 1.5 times the reference doses of RG, GS, GL and AG were prepared and administered twice a day. All of the drugs were given at the concentration of 0.4 ml/20 g. One and a half times the crude drug reference doses of RG, GS, and GL was 2.25 g/kg (0.045 g/20 g), while 1.5 times the crude drug reference dose of AG was 1.5 g/kg (0.03 g/20 g). Therefore, the concentration of RG, GS, and GL was 0.1125 g/ml, and the concentration of AG was 0.075 g/ml. The doses of RG, GS, GL and AG were prepared each day for administration.