Brief Report

Serum fructose concentration in rats after single dose oral administration of Si-Wu-Tang

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ABSTRACT: Our previous study showed that fructose is an important active constituent that is responsible for Si-Wu-Tang's (SWT) effects promoting hematopoiesis and immunity. In order to provide primary data for analysis of the mechanism of fructose's bioactivity, the concentration of serum fructose in rats after a single oral administration dose of Si-Wu-Tang was determined. The concentration of serum fructose in fasting rats was 0.34 ± 0.24 mg/dL. After oral administration of 7.2 mL per kg body weight of SWT extract (1 mL extract corresponds to 1 g SWT dried herbs), serum fructose levels reached a peak concentration of 1.03 ± 0.25 mg/dL within 60 min, and then declined to the baseline level within 180 min, a pattern which is similar to the one reported for oral administration of pure fructose. The peak concentration was only 2-3 times higher than the baseline serum fructose concentration. These results showed that the increase of blood fructose concentration after oral administration of SWT is small and transient, which is very probably due to the quick metabolism of fructose by the liver. We suggest, for future research, it is necessary to consider the probability that fructose's bioactivity on hematopoiesis and immunity is not exerted by fructose in its original form, but after it is metabolized by the liver.

Keywords: Blood, concentration, fructose, rat, serum, Si-Wu-Tang

1. Introduction

Si-Wu-Tang (SWT), a traditional Chinese formula consisting of *Rehmanniae Radix*, *Angelica Radix*, *Chuanxiong Rhizoma* and *Paeoniae Radix*, has traditionally been used in China for about one thousand years (*1*). Dai *et al*. reported that SWT has been used for the treatment of gynecologic diseases (*e.g.* dysmenorrhea, menoxenia, metrorrhagia, abortion), cutaneous diseases (*e.g.* pruritus, urticaria, eczema, dermatitis), and chronic inflammation (*e.g.* chronic nephritis, pelvic inflammation) (*2*). It has been reported to possess sedative, anti-coagulant and antibacterial activities and to exhibit effects of vasodilatation, hematopoiesis, enhancement of cellular immunity and radio-protection (*3,4*). Our interest has been focused on SWT's hematopoiesis-related activities. Using 3.5 Gy ^{60}Co γ-ray irradiated mice as a model of anemia, we found that SWT increases the number of peripheral leukocytes and four types of progenitor cells in bone marrow, colony-forming unit-granulocyte-macrophages (CFU-GM), colony-forming unit-mature erythroid (CFU-E), colony-forming unit-immature erythroid (BFU-E) and colony-forming unit-multipotential (CFU-mix) cells (*5*). In our latest report, fructose was shown to be an important active constituent responsible for SWT's effect promoting hematopoiesis and immunity after oral administration (*6*). Therefore, it has currently become one of the major concerns of our research effort to find out the mechanism of fructose's bioactivity.

To study the mechanism of fructose's bioactivity, changes of blood concentration after SWT administration is primarily required. It will help us to know the characteristics of the absorption, metabolism and elimination process of fructose after oral administration of SWT, which should help us analyze the possible mechanism of fructose's bioactivity. On the other hand, if *in vitro* experiments to study the mechanism of fructose's bioactivity are to be performed in the future, a proper concentration of fructose will be required. In the present study, we investigated the changes of serum fructose concentration in rats after oral administration of SWT for the purpose of providing primary data for the mechanism of analysis of fructose's bioactivity.

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2. Materials and Methods

2.1. *Animals*

Female Sprague-Dawley rats (274 ± 20 g) were purchased from the Experimental Animal Center of the Academy of Military Medical Sciences (Beijing, China). They were housed in an environmentally controlled breeding room with free access to standard animal chow and tap water, and were allowed at least three days acclimatization before an experiment. Each rat was used once and treated in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and was fasted for 24 h before the test.

2.2. *Drugs and reagents*

An extract of SWT was prepared by decocting the dried prescription of herbs with boiling water. After the first decoction the duration of which was about 30 min, the suspension was filtered and water was added for the second decoction of about 20 min. The filtered and mixed suspension from two decoctions was condensed to a concentration of 1 g dried herb weight/mL solution and then stored at – 20°C before administration. The ingredients of 41 g SWT include 15 g of *Rehmanniae Radix*, 10 g of *Angelica Radix*, 6 g of *Chuanxiong Rhizoma* and 10 g of *Paeoniae Radix*. These ingredients correspond to the following plants: *Rehmannia glutinosa* LIBOSCH. (*Scrophulariaceae*), *Angelica sinensis* (OLIV.) (Umbelliferae) DIELS, *Ligusticum chuanxiong* HORT. (Umbelliferae), and *Paeonia lactiflora* PALL (Paeoniaceae), respectively. These plant materials were purchased from Tongrentang, Ltd. (Beijing, China) and identified by Dr. Baiping Ma in our laboratory. Fructose was purchased from Sinopharm Chemical Reagent Co., Ltd. Glucose, sucrose, potassium carbonate and anhydrous magnesium sulfate were purchased from Beijing Chemical Reagent Company. FA-20 fructose assay kit, glucose oxidase and catalase were purchased from Sigma-Aldrich Co., St. Louis (MO, USA). Perchloric acid was purchased from Jinlu Chemical Co., Ltd. (Shanghai, China).

2.3. *Content of fructose in SWT*

To calculate the administered dose of fructose, the contents of fructose and sucrose in SWT were quantitatively determined using our previously reported high performance liquid chromatography (HPLC) method (*7*) for simultaneous determination of fructose, glucose, and sucrose, with slight modifications. Briefly, 1 mL of the SWT extract was diluted to 100 mL with water and then added to 400 mL ethanol and filtered to remove precipitation. The filtered solution was evaporated and solubilized with 50 mL of 50% ethanol solution. Two μL of this solution was injected into an Angilent 1100 HPLC system with a SHISEIDO carbohydrate column (5 μm, 150 × 2.0 mm) and a PL-ELS 2100 evaporative light scattering detector (ELSD) for analysis. The mobile phase was isocratic acetontrile and water (75:25, v/v). The flow rate was 0.2 mL/min. The evaporation temperature, nebulization temperature and gas flow of ELSD were 25°C, 25°C, and 1.0 mL/min, respectively. The contents of fructose, glucose, and sucrose in the SWT extract were determined to be 41.6, 37.5, and 92.4 mg/mL, respectively, using an external standard method.

2.4. *Collection and measurement of blood samples*

Each rat was administered a single oral dose of 7.2 mL per kg body weight of SWT extract (1 mL extract corresponds to 1 g dried herbs of SWT). At time zero and at times of 15, 30, 60, 90, 120, 180, 240, 360, 540 min after dosing, a blood sample (3-5 mL) was collected from the aorta of the rat under anesthesia. Within 60 min after blood withdrawal, the samples were centrifuged and the separated serum samples were frozen in polypropylene tubes at – 20°C prior to analysis. Following the procedure described by Beuter HO (*8*), the serum samples (1.5-2.5 mL) were deproteinized with an equal volume of 0.6 M perchloric acid solution. The concentrations of fructose were then measured with the FA-20 fructose assay kit. In this method, hexokinase, phosphoglucose isomerase, and glucose-6-phosphate dehydrogenase are used and the increase in absorbance at 340 nm is directly proportional to fructose concentration. Data were expressed as mean ± standard deviation (mean ± S.D.).

3. Results and Discussion

All experimental subjects were female Sprague-Dawley rats (274 ± 20 g). All rats orally received a single dose of 7.2 mL SWT extract per kg body weight. One mL extract corresponds to 1 g dried herbs of SWT and contained 41.6 mg fructose and 92.4 mg sucrose (determined by HPLC). Fructose in both free monosaccharide form and disaccharide form (sucrose) in 1 mL SWT extract was calculated to be 90.2 mg (41.6 + 92.4 × 180/342 = 90.2). As shown in Figure 1, fructose concentration in fasting rat serum was 0.34 ± 0.24 mg/dL. The peak rat serum fructose concentration after the single dose of SWT was 1.03 ± 0.25 mg/dL. This was reached within 60 min and then declined to baseline levels within 180 min.

This is the first report concerning blood fructose concentrations after SWT oral administration. Fructose was shown in our previous research to be an important active constituent responsible for SWT's effect promoting hematopoiesis and immunity. To analyze the possible mechanism of fructose's bioactivity, we

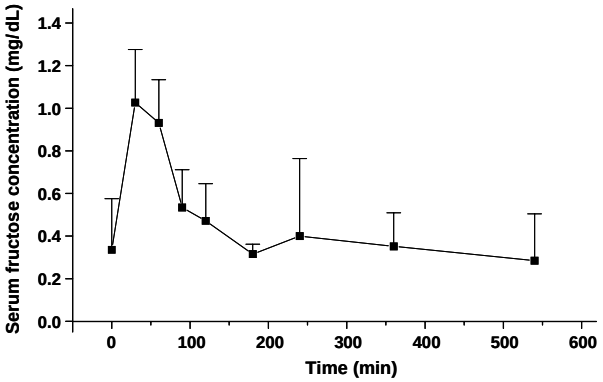

Figure 1. Concentration of serum fructose after SWT administration in rats. Each rat was administered a single oral dose of 7.2 mL per kg body weight of SWT extract (1 mL extract corresponds to 1 g dried herbs of SWT). Data represent means ± S.D.

need blood fructose concentration data after SWT administration. To be consistent with our previous studies on SWT's bioactivity performed on female mice, an equal dose was used in this study of rats, which was converted from mice (calculated from the human clinical dose) to rats through normalization of the body surface area (BSA), and the gender of rats was also female. SWT contains a large amount of fructose, in both free monosaccharide form (*i.e.,* fructose) and disaccharide form (*i.e.,* sucrose). Sucrose is hydrolyzed by sucrase in the digestive tract after oral consumption and each molecule of sucrose produces one molecule of fructose and one molecule of glucose. In this study, the contents of fructose and sucrose in the SWT extract were determined to be 41.6 and 92.4 mg/mL, respectively. Therefore, the total fructose content (in both forms) in 1 mL SWT extract was about 90.2 mg, and the single dose of 7.2 mL/kg SWT extract received by the rats approximately corresponds to a fructose load of 0.65 g/kg. If extrapolated to the human equivalent dose through normalization to BSA, it approximately corresponds to a fructose load of 0.14 g/kg for human.

The results showed that the increase of blood fructose concentration after a single oral administration dose of SWT was very small. The maximum serum fructose concentration was only 2-3 times higher than the baseline level, which occurred within 60 min. These patterns were similar to reported patterns of blood fructose concentration after a single oral dose of pure fructose. Reportedly the concentration of fructose in fasting blood of healthy humans is typically 1 mg/dL or less, and after oral administration of a fructose load in doses ranging from approximately 18 g (0.25 g/kg of body weight) to 100 g, the mean plasma or serum fructose concentration increased to values ranging from 4.5-13.0 mg/dL. Peak fructose concentrations were seen 30-60 min after fructose ingestion (*9*). The study on rats showed that after oral pure fructose consumption (6.9 μmol/g), the plasma fructose concentration of rats reached its peak value at about 60 min (*10*). Similarly in our study, the concentration of serum fructose in fasting rats was 0.34 ± 0.24 mg/dL. After oral administration of SWT extract, serum fructose levels reached a peak concentration of 1.03 ± 0.25 mg/dL within 60 min, and then declined to baseline levels within 180 min. Such a small and transient increase of blood fructose concentration after SWT administration may be explained by the quick and efficient metabolism of fructose by the liver. According to Havel's review (*11*), after consumption of moderate amounts of fructose, the absorbed fructose arrives at the liver *via* the portal vein and is efficiently taken up by the liver such that little escapes hepatic metabolism and enters the systemic circulation. A 1 g/kg oral dose of fructose results in a blood fructose level of only 9 mg/dL. In fact the total dose of fructose received by the rats in our study was even smaller than the "moderate amounts" mentioned in Havel's review, which was only about 0.14 g/kg when extrapolated to the human equivalent dose.

Generally, there are two hypotheses for mechanisms by which the fructose in SWT may help the body's hematopoiesis and immunity. The first one is that the fructose, after entering the blood circulation, acts directly on a certain target (*e.g.* the bone marrow cells) in its intact monosaccharide form. The other one is that the fructose produces its effect after it is metabolized by the body. Given the reported feature of quick metabolism of pure fructose from oral administration as well as the results confirmed in our experiment with SWT oral administration and suppose that fructose helps the body's hematopoiesis and immunity. If the fructose acts directly on a certain target in its intact monosaccharide form, it does not seem very convincing that fructose could produce a remarkable biological effect with such a small and transient increase in blood concentration. Therefore we suggest, for future research, it is necessary to consider the probability that the fructose helps the body's hematopoiesis and immunity, not in its original form, but after it is metabolized by the body.

The liver is the primary metabolic site of fructose disposal. According to Owen's review (*12*), three factors contribute to this: (*i*) enzymes essential for the metabolism of fructose, fructokinase, and triokinase are highly expressed in the liver; (*ii*) the liver is exposed to higher concentrations of orally administered fructose than other tissues; and (*iii*) the high first pass extraction of fructose by the liver limits the availability of fructose for metabolism by peripheral tissues. Current knowledge of fructose's metabolism has shown that, in the liver, fructose is metabolized by fructokinase to fructose-1-phosphate that is cleaved by aldolase B to form dihydroxyacetone phosphate and glyceraldehyde, both of which can be further metabolized in the glycolytic pathway (*13*). On the other hand, it was reported that low-dose fructose (infused into the duodenum) could increase hepatic

glucose uptake and glycogen storage, which is possibly due to the activation of glucokinase by a trace amount of fructose acting on the glucokinase regulatory protein (*14*). Stimulating effects on insulin-stimulated hepatic glycogen synthesis from low-dose fructose were also reported (*15*). Therefore, it is necessary to consider the possibility that fructose may help the body's hematopoiesis and immunity by participating in and/or regulating glucose metabolism. In our previous studies on SWT and fructose's bioactivities, the model of anemia was induced by γ-ray radiation. Interestingly, according to Fang Y (*16*), a higher intake of energy is required by irradiated experimental animals because radiation may: (*i*) inhibit the oxidation phosphorylation process and lead to a low P/O ratio; (*ii*) affect Krebs cycle and decrease production of NADH and $FADH_2$ which are materials for the oxidation phosphorylation process; and (*iii*) increase the basal metabolic rate. The lack of energy or nutrition increases the body's sensitivity to radiation, and experiments showed that an enhanced energy supply prevents dogs from loss of body weight after irradiation. According to Fang, radiation decreases the activity of hexose kinase and thereby interrupts the transformation from glucose to glucose-6-phosphate, which is the first step of the glycolytic pathway. However, the transformation from fructose to fructose-1-phosphate is unaffected because the activity of fructokinase is not changed by radiation. The experiment demonstrated that among four different sugars (sucrose, dextrin, cornstarch, and glucose), glucose showed the most significant therapeutic effect against radiation injury, but when comparing the therapeutic effect of glucose with fructose, fructose was even better. Therefore, a possible explanation for the mechanism of fructose's bioactivity is that fructose may help the body's hematopoiesis and immunity by improving the body's carbohydrate metabolism or energy supply which is relatively insufficient in irradiated animals. Further studies concerning the relationship between fructose metabolism as well as carbohydrate metabolism and radiation injury are required.

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