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Multiple toxicity studies of trehalose in mice by intragastric administration

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ABSTRACT

In the present study, aberration, body weight, food consumption, haematology, organ coefficients, and both gross and microscopic appearance of some histiocytes were compared between the test and control groups. A sperm abnormality test, bone marrow cell micronucleus test, and a haematology study were conducted at levels of 1.25 g/kg, 2.5 g/kg, and 5 g/kg of trehalose. In both the sperm abnormality test and bone marrow cell micronucleus test, statistically significant differences were observed between the positive control and treatment groups ($P < 0.05$), while no statistical difference was observed among the negative control, high dose, moderate dose and low dose groups ($P > 0.05$). In the haematology study, there was no significant difference found from the controls at $P > 0.05$. The results obtained in the present study could support the conclusion that consumption of trehalose has no adverse effects for humans.

1. Introduction

Trehalose (O-α-D-glucopyranoside-(1→1)-α-D-glucopyranoside) is a non-reducing disaccharide that exists in a large range of organisms, such as microorganisms, plants, and insects. Furthermore, beer yeast can accumulate large amounts of trehalose (up to 15% of the dry weight) during the stationary growth phase in some extreme environments (Lillie & Pringle, 1980). Recently, trehalose has been highly valued because it can be used as an excellent bio-protectant (Gibson et al., 2008). The mechanisms by which trehalose protects biologic molecules can be divided into three categories, namely water replacement, glass transformation and chemical stability (Colaco & Roser, 1995). Trehalose has many biological functions, not only as a storage carbohydrate and transport sugar, but also has an important role in stress protection, especially during heat stress and dehydration (Crowe, Carpenter, & Crowe, 1998; Wiemken, 1990). Additionally, trehalose can protect animals, plants, and microorganisms from severe environmental damage, such as nutrition deficiency, freezing, hyperosmosis, saline stress, and dehydration, as an emergency metabolite (Streeter, 2003; Voita, 2003). It has been argued (Aldous, Alffret, & Franks, 1995) that trehalose has excellent ability to stabilize proteins and peptides, due to the fact that it can absorb water, then crystallize, and subsequently phase separate from the protein/peptide. Moreover, trehalose has a strong stabilizing effect on biological structures by forming a glasslike structure after dehydration. Trehalose is predicted to become a useful stabilizer in foods and an additive in cosmetics and pharmaceuticals because of these characteristics (Colaco et al., 1994; Roser, 1991). Trehalose has industrial significance in food, pharmaceutical, cosmetics and many other industries (Elbeinm, Pan, & Pastuszak, 2003).

When ingested, trehalose is enzymatically hydrolyzed in the small intestine by a trehalose-specific disaccharidase into two D-glucose molecules. The physiological processes are the same as other common disaccharides like maltose and sucrose (Dahlqvist, 1974). It has been shown that trehalose could improve the membrane integrity of mouse fibroblasts during drying when presented both in vivo and in vitro (Chen et al., 2001). Furthermore, the concentration of intracellular trehalose up to 0.15 M does not impair the development of mouse zygotes. Interestingly, this concentration is within the range of effective sugar concentrations found in anhydrobiotic organisms (Ali, Joel, Mehmet, & Thomas, 2003). Trehalose showed no genotoxicity in standardized Ames or clastogenic assays, even at the highest concentrations tested (5000 μg per plate). Richards and colleagues (2002) fed mice and rabbits with high concentrations of trehalose in chromosome aberration, acute oral toxicity, embryotoxicity and reproductive studies; however, no adverse effects were observed in any of the studies. In this study, the sperm abnormality test was first used to detect reproductive toxicity. Additionally, clinical observations, haematology, serum biochemistry indicators, organ coefficients and

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histopathology were all performed in this study. The objective of this research was to investigate toxicological effects of trehalose in mice using a variety of tests, as well as to explore its possible application as a value added product.

2. Materials and methods

2.1. Materials and reagents

Trehalose was purchased from Sigma Chem. Co. (St. Louis, MO, USA). Granulous diets were purchased from Experimental Animal Center, Jilin University, and were in conformity with Chinese standard GB 14924-2001. Ethylenediamine tetraacetic acid-2K (EDTA), and all the other materials required in the experiments were purchased from Beijing Chemical Plant (Beijing, China).

2.2. Instruments and equipment

A MEK-6318K fully automatic hemocytometer produced by Nihon Kohden Company (Nihon Kohden Co., Ltd., Tokyo, Japan) was used for counting cells. A high-speed freeze centrifuge (CR20B2) produced by Hitachi Company (Hitachi Koki Co., Ltd.). A LC-2010 High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan), equipped with a Sugar-Pak 1 Column (6.5 mm × 300 mm) (Waters Ltd., America) and RID-10A refractive index detector (Shimadzu, Japan), and a CR20B2 high speed freezing centrifuge (Hitachi Ltd., Shanghai, PR China) were used.

2.3. Determination of trehalose content by HPLC

The HPLC was equipped with a Sugar-Pak 1 Column (6.5 mm × 300 mm) and RID-10A detector. The mobile phase was EDTA aqueous solution of 50 mg/ml at a flow rate of 0.5 ml/min, and the column temperature was 90 °C. A trehalose standard solution of 1 mg/ml for HPLC determination with injection volumes of 10 μl was used to determine the trehalose content.

2.4. Animal care and groups design

One-hundred-forty healthy male mice weighing 18–20 g were obtained from the Experimental Animal Center, Jilin University, at the beginning of the experiment. All animal experiments were conducted in compliance with the Guide of The Care and Use of Laboratory Animals (National Research Council, 1996). All mice were randomly allocated to the respective experimental groups, and were housed in polypropylene cages (320 × 215 × 170 mm) with stainless steel covers. The animals were maintained at a room temperature of 22 ± 2 °C, relative humidity of 30–70% and ventilation frequency of ⩾10 times/h; room lights were on for approximately 12 h per day. The animals were fed with a standard diet (3883.0.15, Provimi Kliba SA, Kaiseraugst, Switzerland) and supplied running water available ad libitum. All toxicological studies complied with the Animal Welfare Act (USDA, 1985). All mice were acclimatized for 7 days prior to the experiment, in which there were no trehalose-related changes in general states, including body weight, food-intake, skin, excretions, gait, and behaviour.

The toxicity assessment experimental group designation is shown in Fig. 1. All animals were given intragastric administration and were fasted overnight before lethal exarticulation. The mice were randomly allocated into 14 groups for three tests. Five groups (n = 10 mice in each group) were prepared for the bone marrow cell micronucleus test. The mice of the positive control group were perfused orally with cyclophosphamide (CP) at doses of 40 mg/kg, and the mice of negative control group were given solvent (distilled water) at the same doses. Compared to the animals in the control groups, the animals of low, middle and high dose groups were given 1.25 g/kg, 2.5 g/kg, and 5 g/kg trehalose by intragastric administration respectively (Richards et al., 2002). The five groups (n = 10 mice in each group) were exposed continuously for 3 days (Gao, Ye, Yu, Zhang, & Deng, 2011). Moreover, other five groups were (n = 10 mice in each group) were prepared for the sperm abnormality test in which all animals were grouped and treated under the same conditions as the bone marrow cell micronucleus aberration test. However, these groups were exposed continuously for 5 days, while observations were continued for 35 days. The remaining four groups (n = 10 mice in each group) were prepared for the haematology study which included: haematological examination, organ coefficients, food intake and weight changes. The mice in the negative control group were given solvent (distilled water) at the 40 mg/kg doses. All of the mice were fed with the pathognostic diet for 40 days.

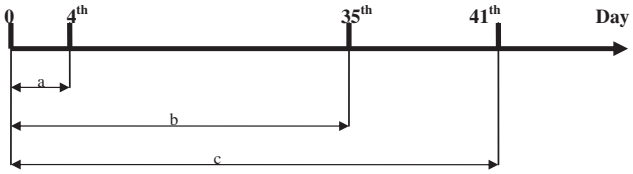

Fig. 1. Animal studies design on safety assessment of trehalose. (a) Bone marrow cell micronucleus aberration test: Five groups were used for the test (n = 10). All animals were exposed continuously for 3 days. On the 4th day, all the mice were killed by cervical dislocation. (b) Sperm abnormality test: Five groups were exposed continuously for 5 days. The observations were continued for 35 days. On the 36th day, the mice were killed by exarticulation. (c) Haematology study: it contains haematological examination, study on organs and study on consumption and weight. Four groups' male mice were used for these studies. The mice were fed with the pathognostic diet for forty days. On the 41th day, the mice were killed by exarticulation.

2.5. Bone marrow cell micronucleus test

On the 4th day, all the mice were killed by dislocation of cervical spine. The breastbone marrow was sliced and observed under an oil immersion lens (Motic, 282AQG-M5, USA). The genotoxicity was evaluated by measuring the micronucleus rate of 1000 polychromatic erythrocyte (PCE) cells in bone marrow.

2.6. Sperm abnormality test

The experimental design was based on Chinese toxicology guideline GB15193.13-2003. Briefly, on the 36th day, the mice were killed by dislocation of cervical spine, and then two epididymes of each mouse were removed and cut into pieces with surgical scissors in 1.5 ml physiological saline. Thereafter, the sperm suspension was filtered out using three-tiers of lens cleaning paper. A drop of the filtrate suspension was coated evenly on a glass slide with a glass rod. After air drying, the cells were fixed with formaldehyde for 10 min, and stained with eosin. The sperm abnormalities were enumerated by the methodology of Hemavathi and Rahiman (1993) using light microscope (Motic, 282AQG-M5, USA).

2.7. Haematology study

2.7.1. Haematological test

Haematology is often used to detect physiological changes following different stress conditions. On the 41st day, prior to necropsy, the blood samples for haematology and clinical biochemistry evaluations were collected via the abdominal aorta under anesthesia. Approximately 500 μl of blood samples were collected in EDTA-2K coated tubes and used for haematological