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A 13-week subchronic oral toxicity study of L-serine in rats

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ABSTRACT

A subchronic oral toxicity study was conducted to evaluate the safety of L-serine in Sprague–Dawley rats. The test article was administered once daily by gavage in male and female rats at dose levels of 0, 500, 1500, and 3000 mg/kg body weight/day for 13 weeks. Daily clinical signs, body weight, and food consumption were not affected by ingestion of the test article. There were no treatment-related adverse effects on urinalysis, hematology, serum biochemistry, organ weights, gross and histopathological examination. It was concluded that the no-observed-effect level (NOEL) for L-serine was 3000 mg/kg bw/day for both genders.

1. Introduction

Amino acids are mostly supplied in the normal diet not as free amino acids but rather as protein constituents. Moreover, they have been taken as food additives in addition to normal dietary intake of protein. The safety of amino acids consumed from the diet has not been of great concern because they are nutrients required for the synthesis of functional and structural components of the body and are consumed in large quantities from food as an essential part of the diet. In recent years, there has been growing interest in the safety of individual amino acids due to a large increase in the consumption of individual amino acids therapeutically or as dietary supplements for supporting pharmacological actions or enhancing health or physical performance.

Some of the effects of disproportionate intake of amino acids have been reported (Harper et al., 1970; Benevenga and Steele, 1984), and these reviews have showed that a growth depression is induced when certain single amino acids are given in excess to growing animals. However, this event develops only in growing animals fed low-protein diets. There have been numerous literature reports regarding the safety of some amino acids used as food additives such as glutamate, aspartate, and phenylalanine (Anderson and Raiten, 1992). There have been relatively few studies regarding the toxicity of arginine, glutamine, and branched-chain amino acids, which are used as drugs or dietary supplements, although have been many studies attempting to determine their clinical and physiological benefits, with adverse effects having been noted. Fewer studies have been carried out regarding the safety of many other amino acids (Garlick, 2004).

L-serine, one of the nonessential amino acids, is biosynthesized from an intermediate of the glycolytic system and is a precursor for the synthesis of other amino acids (glycine and L-cysteine), lipids (phospholipids and sphingolipids), and nucleotides. In addition, L-serine has been implicated as a neurotrophic factor (Mitoma et al., 1998; Furuya et al., 2000). We have recently found that intracerebroventricular injection of L-serine has sedative and hypnotic effects under an acute stressful condition in neonatal chicks (Koutoku et al., 2005; Asechi et al., 2006) and it appears that these effects occur by enhancing inhibitory neurotransmission via $GABA_A$ receptors (Shigemi et al., 2008). It is well known that activation of $GABA_A$ receptors favors sleep. A lot of hypnotics are based on $GABA_A$ receptor-mediated inhibitory processes (Gottesmann, 2002). A few food materials which exhibit their effects via $GABA_A$ receptors were reported. It was shown that $GABA_A$ receptors mediated the hypnotic activity of melatonin (Wang et al., 2003), and they were major cellular substrate for the anxiolytic action of valerian extracts (Benke et al., 2009). These results suggested that L-serine may be effective in improving anxiety or sleep disorders induced by psychological stressors. It is likely to be useful as an alternative medicine as an antianxiety agent or sleep-inducer if it can be confirmed to be safe.

L-serine has been used as a food additive, but it has never been used therapeutically or as dietary supplement. There is therefore little information regarding its safety. In animal studies, the administration of DL-serine has been found to induce renal necrosis (Morehead et al., 1945), but not L-serine (Artom et al., 1945). Serine (isomer not specified) given to animals lowered blood pressure and heart rate, however serine was injected intracisternally in the study (Takemoto, 1991). In human studies, no effects were reported in 4 healthy control subjects given a single oral dose of 210 mg/kg of serine (Pepplinkhuizen et al., 1980). There is a report

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indicating that 3 doses of 5 g of L-serine (190 mg/kg) every day for approximately 12 weeks were well-tolerated, but the subject was a pregnant woman who had a 3-phosphoglycerate-dehydrogenase deficient fetus (de Koning et al., 2004). Thus both animal and human studies reported to date provide few data regarding the effects of long-term intake of L-serine.

A 13-week repeated oral dose toxicity study in rats was conduced to evaluate the safety of excessive intake of L-serine. The present study was conducted in compliance with Technical Specifications for Examination and Assessment of Health Food as set forth in the Ministry of Health of the People's Republic of China, issued 2003, which meet OECD guidelines.

2. Materials and methods

2.1. Test article

L-serine, L-2-amino-3-hydroxypropionic acid, CAS Number 56-45-1, was supplied as white crystalline powder and was stored at room temperature. The purity was 99.2%, as determined by HPLC. L-serine was dissolved with distilled water to concentrations of 50, 150, and 300 mg/ml to provide administered doses of 500, 1500, and 3000 mg/kg bw/day. Test articles were prepared twice a week and packaged at daily doses and stored at 4 °C before use.

2.2. Animals

Male and female Sprague–Dawley rats at 5 weeks of age purchased from Beijing Weitonglihua experimental animal technique Co. Ltd (Beijing, China) were used. The animals were acclimated to housing conditions for 1 week, and the treatment started at 6 weeks of age to healthy animals without abnormalities suggestive of health problems. The animals were kept in a room maintained at a temperature of 20–22 °C and a relative humidity of 40–70% with a 12-h light/12-h dark cycle and with approximately 15 times air changes per hour. Five animals were housed in a bottom-meshed stainless cage. The animals were allowed filtered tap water and the fixed-formula rat granula feed (Beijing Keaoxieli forage Co. Ltd., Beijing, China) ad libitum.

2.3. Experimental design

Animals were randomly divided into four groups: three treatment groups of L-serine 500, 1500, and 3000 mg/kg and a control group. Each group consisted of 10 males and 10 females. The test articles were administered daily by gavage to rats for 13 weeks at dose levels of 500, 1500, and 3000 mg/kg, and the control rats received distilled water alone. Individual dose volume of 10 mL/kg was calculated according to the latest measured body weight. The animals were observed daily for clinical signs and mortality, and body weights were measured twice a week during the study period. The amounts of supplied and residual diet were weighed twice a week in order to calculate the daily food consumption.

2.4. Urinalysis

During the last 4 days of administration period, urinalysis was conducted in all rats. Animals were individually placed in metabolic cages, provided with water and deprived of diet. The urine was collected for 24 h, and the volume was measured. The analysis of urine pH, protein, glucose, occult blood, specific gravity, urobilinogen, nitrite, ketone body, bilirubin, and leukocytes was conducted by using urinary test papers in the Urine Analysator (Bayer 50, Germany). The analysis of sodium, potassium and chloride was performed by using an electrolyte analyzer (Easy Lyte PLUS Na/K/Cl ANALYZER, Medica Co., USA).

2.5. Hematology and serum biochemistry

After the treatment, all animals were fasted overnight and then anesthetized by phenobarbital sodium, and blood samples were collected from the abdominal aorta. The following parameters were measured: red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), Hematocrit (Ht), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leukocyte differential count were analyzed by using an automatic blood cell analyzer (MEK 6318 K, Nihon Kohden Co. Ltd., Japan). The reticulocyte count (Ret) was carried out with the blood smears stained with brilliant cresol blue. The following parameters for blood biochemistry using serum after centrifugation of whole blood: total protein (TP), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total bilirubin (T-bil), total cholesterol (T-cho), triglyceride (TG), calcium (Ca), and inorganic phosphate (IP) were determined with an automatic biochemistry analyzer (Dade Dimension AR, Block scientific Inc., Germany). Serum electrolytes such as sodium (Na), potassium (K), and chloride (Cl) were measured with an electrolyte analyzer (Easy Lyte PLUS Na/K/Cl ANALYZER, Medica Co., USA).

2.6. Histopathological assessment

The animals were sacrificed by exsanguinations from the abdominal aorta. At necropsy, the brain, pituitary, salivary glands, thymus, lung (including bronchi), heart, liver, spleen, kidneys, adrenal glands, testes, prostate, seminal vesicle, ovaries, and uterus were removed and weighed. The relative organ weights were calculated using the animals' fasted body weights. In addition, the spinal cord, thyroid glands, optic nerve, eyes, stomach, small intestine (duodenum, jejunum and ileum), large intestine (cecum, colon and rectum), pancreas, mesenteric lymph nodes, urinary bladder, epididymides, and bone marrow were also removed. All organs and tissues were examined grossly and then fixed in 10% buffered formalin solution. Histopathological assessment was first performed on all tissues of the control and highest dose groups for both sexes. If any treatment-related changes appeared at

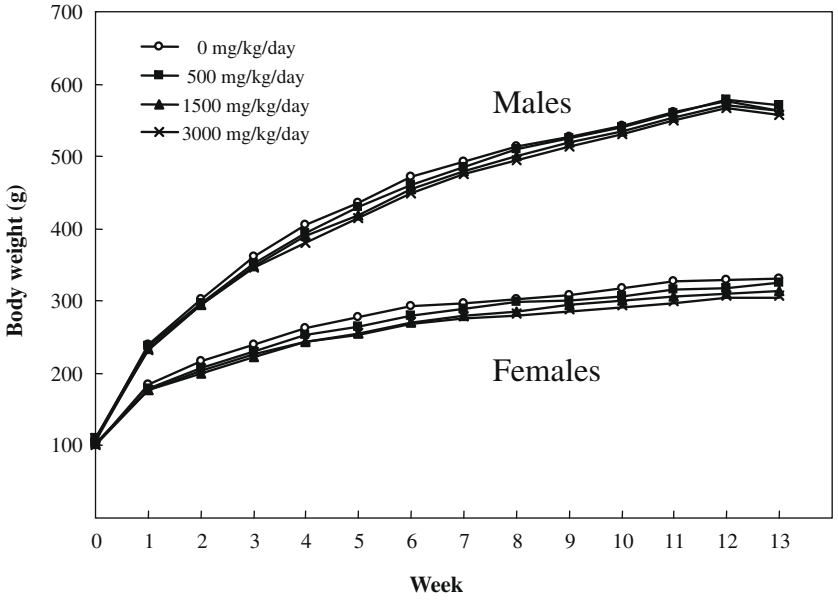

Fig. 1. Body weight curves for male and female rats administered with L-serine for 13 weeks.