Food and Chemical Toxicology 46 (2008) 2789–2795

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Toxic effects of L-aspartic acid at high dose levels on kidneys and salivary glands in Fischer 344 rats detected in a 90-day feeding study

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ARTICLE INFO

Article history:
Received 6 December 2007
Accepted 13 May 2008

Keywords:
L-Aaspartic acid
Subchronic toxicity test
Feeding study
Renal toxicity
Salivary glands toxicity
Fischer 344 rat

ABSTRACT

A subchronic oral toxicity study of L-aspartic acid (L-Asp) was conducted with groups of 10 male and 10 female Fischer 344 rats fed a powder diet containing 0%, 0.05%, 1.25%, 2.5% and 5.0% concentrations for 90 days. Serum biochemistry showed treatment-related decreases of blood urea nitrogen, creatinine and uric acid levels in both sexes. In addition, incidences of urinary ketone and protein were significantly increased in treated both sexes, while relative kidney weight was significantly increased in the 5.0% male rat, and regenerative renal tubules with tubular dilation were histopathologically observed in male rats of the 2.5% or greater groups. The observed renal injury was confirmed not to be due to accumulation of α2u-globulin. Acinar cell hypertrophy of salivary glands was histopathologically evident in male and female rats of the 2.5% or greater groups. The present results indicate that L-Asp causes toxic effects on kidneys and possibly salivary glands at high dose levels in male and female Fischer 344 rats. Such toxic effects were observed only in animals given 2.5% and/or higher doses of L-Asp.

In conclusion, the no-observed-adverse-effect-level (NOAEL) for L-Asp is 1.25% (696.6 mg/kg body weight/day for males and 715.2 mg/kg body weight/day for females) under the present experimental conditions.

1. Introduction

Risk assessment and management of chemicals present in or introduced into the human environment are really important to maintain human health and welfare. Each chemical therefore needs to be assessed very carefully and strictly according to applicable laws, regulations and rules provided both domestically and internationally. The potential risk to human health of food additives is, needless to say, a crucial social as well as regulatory issue. In Japan, the Ministry of Health, Labour and Welfare (MHLW) has responsibility for risk assessment and management of these chemicals in collaboration with the Food Safety Commission. In 1947, MHLW enacted the Food Sanitation Law as the first comprehensive law for food safety/hygiene and introduced a positive list system for food additives. Under the system, only additives approved as safe by MHLW may be used in foods. Although all food additives were considered covered for regulation by this law, in reality, the designation system was applied only to chemically synthesized additives until 1995, when the Food Sanitation Law was amended. After the amendment of the law, however, all types of additives, both synthetic and non-synthetic, have been equally treated in the designation system, with minor exceptions of so-called existing food additives, natural flavoring agents and substances that are generally provided for eating or drinking as foods and used as food additives. Existing food additives are defined as substances that were already marketed or used on the date of the amendment of the Food Sanitation Law in 1995. They must be put on the List of Existing Food Additives that has been released and updated by MHLW, and there were 418 items on the list as of September 11, 2007 (List of Existing Food Additives, 2007). According to the law, the risks of items approved as existing food additives have not been necessarily assessed, but of course various parties have claimed the necessity of their risk assessment. In response, MHLW has been granting research funds to deal with this issue by assessing risks of items on the list, and the present study was conducted as a part of this effort.

L-Aspartic acid ((S)-1-amino-1, 2-ethanedicarboxylic acid; L-Asp) [CAS No. 56-84-8] is classified as an acidic amino acid, together with glutamic acid. In Japan, L-Asp is approved as one of the existing food additives for seasoning of the diet (List of Existing Food Additives, 2007). Recently, L-Asp has become widely used as an ingredient of supplements, health foods and cosmetics. There are only few reports available, however, regarding toxicity of L-Asp. Both rat oral and rabbit dermal median lethal doses (LD_{50}s) were determined to be 5000 mg/kg body weight (Material Safety Data Sheet, 2005), but another report described the oral LD_{50} for rats to be >16 g/kg body weight (Huntingdon Research Centre, 1971). In addition, the intraperitoneal LD_{50} for mice was reported to be 6 g/kg body weight (RTECS). Muramatsu et al. (1971) demonstrated depression of body weight gain in Donryu rats given

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doi:10.1016/j.fct.2008.05.013

L-Asp. Schainker and Olney (1974) suggested that aspartic acid was neurotoxic, causing a relative lack of neurons in the arcuate nucleus in infant mice. In his review about the absorption, utilization, and safety of aspartic acid, Stegink (1976) described that the administration of large quantities of glutamate and aspartate to the newborn mouse produces a variety of neurotoxic effects. Meldrum (1993), however, indicated that the typical dietary consumption of glutamate or aspartate does not cause neurotoxicity in man. On the other hand, dietary administration of D,L-aspartic acid at a dose of 0.5% for 2–8 weeks significantly increased the thymus weight of C57B1/10 mice (Pipalova and Pospisil, 1980), and Schlenker and Goldman (1988) reported that subcutaneous injection of aspartic acid into male and female rats affects the inspiratory flow rate, tidal volume and frequency of breathing. Information about the potential risk of L-Asp is limited, and, assuming the human exposure situation, it needs to be assessed in an urgent but careful manner by twell-established classic protocols. The present study was therefore conducted to examine influence of L-Asp when administered to male and female rats for 90 days in the diet.

2. Materials and methods

2.1. Ethical considerations

The current study was performed in accordance with the Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives released by MHLW (Eika No. 29, March 22nd, 1996). The experimental protocol was approved by our in-house committee, prior to its execution. The experiment was monitored at every step during experimentation for its scientific and ethical propriety, with strict obedience to the National Institutes of Health Guideline for the Care and Use of Laboratory Animals, Japanese Government Animal Protection and Management Law, Japanese Government Notification on Feeding and Safekeeping of Animals, the Guideline for Animal Experimentation (released by the Japanese Association for Laboratory Animal Science, 1987) and other similar laws, guidelines and rules of domestic and international authorities.

2.2. Test chemical and diet preparation

L-Asp (lot No. 0010663004; purity, 100.2%, by a neutralizing titration method) was generously supplied by Ajinomoto Co., Inc. (Kanagawa, Japan). It was admixed into a modified AIN-93G powder diet (Oriental Yeast Co. Ltd., Tokyo, Japan) to give concentrations of 0% (control), 0.05% (corresponding to the human intake level) (Supli-market, 2007), 1.25%, 2.5% or 5.0% every 4 weeks, the diet composition being shown in Table 1. It should be noted here that the 0.05%-group was set to assess effects of a human-relevant dose and thus should be considered an additional group to the standard three-doses (with ratios of 2) for safety assessment study. In fact, the dose 0.05% is 25-fold lower than its immediate upper dose of 1.25%.

The L-Asp content in all experimental diets was analyzed at their preparation, with actual values of 0.56 ± 0.06, 12.71 ± 0.13, 24.90 ± 0.82 and 51.31 ± 0.75 g/kg diet for the 0.05%, 1.25%, 2.50% and 5.0% doses, respectively. After keeping the 0.05% and 5.0% diets for 15 or 30 days at 4 °C, the contents of L-Asp were found to be well stable with values of 0.54 and 51.34 g/kg diet, respectively.

2.3. Animals and treatments

Totals of 55 male and 55 female specific pathogen-free Fischer 344 (F344/DuCrlCrlj) rats were purchased at 5 weeks of age from Charles River Japan Inc. (Kanagawa, Japan) and acclimatized on the control diet for 1 week before the experimentation. The rats were housed individually in stainless steel cages; kept under the controlled conditions of temperature (22–24 °C), relative humidity (50–60%) and ventilation (more than 10 times/hour) with a 12-hour light/dark cycle; and allowed free access to food and drinking water throughout both acclimation and experimental periods.

After confirming normal health status at the end of the acclimation period, 50 rats of each sex were randomly allocated to five groups each consisting of 10 rats, given the control and experimental diets for 90 days. During the experimental period, the rats were observed daily, and clinical signs and mortality (if any) were recorded. Body weights, food and water intakes were monitored weekly.

Table 1
Composition of a modified AIN-93G diet containing L-Asp

Ingredient	Composition (g/kg dry matter)
β-Cornstarch	629.486-X
Casein (vitamin free)	200.000
Soybean oil (no additives)	70.000
Cellulose powder	50.000
Mineral mix (AIN-93G)	35.000
Vitamin mix (AIN-93G)	10.000
L-Cystine	3.000
Choline bitartrate (41.1% choline)	2.500
tert-Butylhydroquinone	0.014
L-Asp	X

2.4. Animal sacrifice and assessments

At the end of the experimental period of 90 days, all rats were deprived of food (but not water) overnight, and fresh urine samples were obtained to be used in the urinalysis of urobilinogen, occult blood, bilirubin, ketone, glucose, protein, pH and nitrous acid by a test paper (N-multistix, Bayer Medical Ltd., Tokyo, Japan). All rats were then anesthetized by ether and sacrificed by exsanguination after collecting blood samples via the abdominal aorta.

Using the blood samples and the sera prepared from them, hematological and serological examinations were performed. Hematological examination was carried out using an automatic analyzer (Sysmex KX-21NV; Sysmex Co., Hyogo, Japan) for red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit level (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and platelet count (PLT). Differential counts of leukocytes were made by light microscopical observation of smear specimens stained with a routine May–Glünwald–Giemsa protocol. Serum biochemistry determination was performed with a Toshiba automatic analyzer (TBA-120FR; Toshiba Medical Systems Co., Tokyo, Japan) for levels of total protein (TP), albumin (ALB), albumin/globulin ratio (A/G), glucose (GLU), total cholesterol (T-CHO), triglyceride (TG), total bilirubin (T-BIL), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), sodium (Na), potassium (K), chlorine (Cl) and calcium (Ca).

At the terminal sacrifice, complete necropsies were performed on all animals. For each animal, the body weight was determined, and gross observations were made. The brain, thyroids (with parathyroids), heart, spleen, liver, adrenals, kidneys, testes, ovaries and uterus were then excised, and their absolute and relative weights were determined. These organs as well as the pituitary gland, eyes, harderian gland, thymus, nasal cavity, trachea, lungs (including bronchi, fixed by inflation with fixative), salivary glands, tongue, esophagus, stomach, duodenum, jejunum, ileum, caecum, rectum, pancreas, urinary bladder, skin with mammary gland, skeletal muscle, epididymides, seminal vesicle, prostate, preputials, oviducts, vagina, lymph nodes (submandibular and mesenteric), thoracic aorta, sciatic nerve, spinal cord (cervical, mid-thoracic and lumbar), bone (femur and sternum) and bone marrow, Zymbal's gland and all gross lesions of each animal were fixed in 10% neutrally buffered formalin. Paraffin-embedded or frozen sections were then routinely

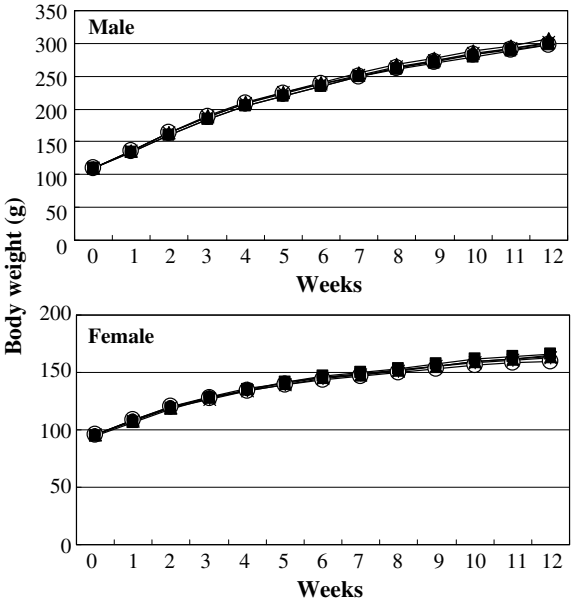

Fig. 1. Weekly changes in average body weights of Fischer 344 rats (10 animals for each group) given L-Asp at dietary doses of 0 (control, open circles), 0.05 (closed triangles), 1.25 (closed circles), 2.5 (asterisks) or 5.0 (closed squares)% for 90 days.