Food and Chemical Toxicology 87 (2016) 55–64

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

13-week repeated dose toxicity study of L-tyrosine in rats by daily oral administration

Yusuke Shibui [a, *], Yasuhiro Manabe [a], Terutaka Kodama [b], Akinori Gonsho [a]

[a] Safety Evaluation Research Group, Fundamental Technology Labs., Institute for Innovation, Ajinomoto Co., Inc., 1-1, Suzuki-cho, Kawasaki-shi, Kanagawa, 210-8681, Japan

[b] Toxicology and Pharmacokinetics Research Group, Development Project Promotion Dept., Research Institute, Ajinomoto Pharmaceuticals Co., Ltd., 1-1, Suzuki-cho, Kawasaki-shi, Kanagawa, 210-8681, Japan

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
16 November 2015
Accepted 21 November 2015
Available online 2 December 2015

Keywords:
L-tyrosine
Amino acid
Toxicity
Rat

ABSTRACT

To evaluate the potential toxicity of L-tyrosine, 4 groups of Crl:CD(SD) rats of both sexes were administered L-tyrosine in water suspension by gavage once daily for 13 weeks at doses of 0 (vehicle), 200, 600 or 2000 mg/kg bw/day. Findings related to L-tyrosine administration were as follows. Edema of the cornified layer at the limiting ridge or forestomach was seen in 600 mg/kg bw/day female group and in both sexes of 2000 mg/kg bw/day group. In the liver, increased weight and hypertrophy of centrilobular hepatocytes were seen in both sexes at 2000 mg/kg bw/day, associated with slight increases in ALT and AST. Regarding the kidney morphology and function, increased hyaline droplets in the proximal tubules and increased urinary protein were seen in the 2000 mg/kg bw/day male group. In addition, increased kidney weight was also observed in both sexes of the 2000 mg/kg bw/day group, although the histological changes attributable to the weight increase remained unclear. As for blood chemistry, increases in triglycerides, total cholesterol, phospholipids, potassium ion, calcium, total protein, and α1 globulin were also seen in both sexes at 2000 mg/kg bw/day. Thus, in this study the no-observed-adverse-effect level (NOAEL) of L-tyrosine was considered to be 600 mg/kg bw/day for males and 200 mg/kg bw/day for females.

1. Introduction

L-Tyrosine is one of the twenty amino acids that compose proteins, and is classified as a non-essential amino acid since L-tyrosine can be generated by biosynthesis from L-phenylalanine in the human body. L-Tyrosine is the precursor and thus essential molecule for the biosynthesis of catecholamine hormones/neurotransmitters, including dopamine, dihydroxyphenyalanine (DOPA), noradrenaline, and adrenaline, in the central and peripheral nervous system and adrenal medulla, and thyroxine and triiodothyronine by the thyroid gland. Also, the melanin pigment (which occurs in the skin, hair, and choroid lining of the eye) forms through enzymatic conversion of L-tyrosine.

L-Tyrosine is listed as a food additive by the Japanese Ministry of Health and Welfare. JECFA (Joint FAO/WHO Expert Committee on Food Additives) and EFSA (European Food Safety Authority) have evaluated that L-tyrosine is acceptable to use as flavouring agent or flavouring substance. There has been only little toxicological information reported regarding L-tyrosine, and therefore, its use has been allowed mainly based on the facts that L-tyrosine is a protein components and nutrient which is taken daily through food in much larger amounts than as a food additive.

It has been pointed out by the International Council on Amino Acid Science (ICAAS) that there is limited toxicological information on some amino acids and further studies are needed. As for L-tyrosine, a small number of toxicity studies has been reported including oral administration in rats (Alam et al., 1966; Muramatsu et al., 1976; Nagaoka et al., 1986) and subcutaneous and intramuscular administration in rats and dogs (Baldrick et al., 2002). However, regarding subchronic oral toxicity of L-tyrosin, the information that can be obtained from the relevant literature is partial and the testing system differs from that employed in today's general toxicological studies (for example, casein diet is used for food and the measurement endpoint is limited). Thus, the toxicity profile of L-tyrosine has not been comprehensively established. In addition, the NOAEL for subchronic oral toxicity of this amino acid

* Corresponding author.
E-mail address: yusuke_shibui@ajinomoto.com (Y. Shibui).

http://dx.doi.org/10.1016/j.fct.2015.11.017
0278-6915/

is not clear from available information. Therefore, we conducted this 13-week repeated-dose oral toxicity study in rats administered L-tyrosine. Repeated oral dose toxicity studies of glycine, L-phenylalanine, L-alanine, and L-threonine have been reported recently (Shibui et al., 2013, 2014., Aoki et al., 2014a,b).

2. Materials and methods

2.1. Study guidelines

This study was conducted in accordance with the OECD Test Guidelines 408 for 'Repeated dose 90-day oral toxicity study in rodents' (OECD, 1998) and the Guidelines for Toxicity Studies of Drugs (Ministry of Health, Labour and Welfare (MHLW), Japan, 1999). Although this study was not conducted under Good Laboratory Practice (GLP) regulations, general procedures in this study were performed according to the standard operation procedures (SOPs) of the GLP compliance testing facility.

2.2. Animals and animal husbandry

Male and female Crl:CD(SD) rats were obtained from Charles River Laboratories Japan, Inc. (Japan) and allowed free access to tap water and a certified rodent diet sterilized by gamma irradiation (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were housed individually in suspended, stainless-steel cages in an animal room at 20–26 °C, relative humidity of 30–70%, and air ventilation of 10–15 times/hour (all-fresh ventilation) under illumination for 12 h a day (from 7 a.m. to 7 p.m.), and acclimatized for 5 or 7 days prior to random allocation. At the initiation of treatment, the animals were 6 weeks old; the males weighed from 176 to 200 g, and the females weighed from 151 to 173 g. All animals were treated humanely according to institutional guidelines, and the experimental procedure was approved by the institutional ethics committee.

2.3. Test article and dosage preparation

L-tyrosine (Cas Number 60-18-4, lot 555012G; L-tyrosine content 99.9%) was supplied by Ajinomoto Co., Inc. (Kawasaki, Japan), and stored at room temperature. Water for injection (lot 3G92N, Otsuka Pharmaceutical Factory Inc., Tokushima, Japan) was used as vehicle and negative control. Concentrations of L-tyrosine suspensions were set at 20, 60 and 200 mg/mL. L-tyrosine suspension was prepared once a day and mixed just prior to each administration. Concentration and homogeneity of L-tyrosine in 20, 60 and 200 mg/mL test formulations at the time of preparation and stability after storage for 1 day at room temperature in 20 and 200 mg/mL test formulations were confirmed (Table 1). Each test items met the acceptance criteria.

2.4. Group designations and treatment

Animals were assigned to treatment groups using a randomized complete block design with body weight stratification by MiTOX computer system (Mitsui Zosen Systems Research Inc., Chiba, Japan). Three groups of 10 males and 10 females were administered L-tyrosine once daily by oral gavage at doses of 200, 600, or 2000 mg/kg bw/day in a dose volume of 10 mL/kg for 91 or 92 consecutive days. Another group of rats (10/sex) was given the vehicle only, and served as the negative control.

2.5. Clinical observation

The animals were observed twice daily (pre-dose and post-dose) for clinical signs including appearance/posture, behavior, feces/urine, body surface, fluid secretion/excretion, and body temperature.

2.6. Body weight

Individual body weight data were recorded on Days 1 and 2, and weekly thereafter.

2.7. Food and water consumption

Individual food and water consumption was measured on Day 2 and weekly thereafter.

2.8. Ophthalmology

Ophthalmic examination was performed on Day 86. The pupils were dilated with a mydriatic agent (Mydrin®-P ophthalmic solution, Santen Pharmaceutical Co., Ltd., Osaka), and the anterior

Table 1
Summary of concentration, homogeneity and stability of L-tyrosine in water suspensions.

Nominal concentration (mg/mL)	Results	Measured concentration (mg/mL)		Judgment*
		Preparation day	After storage for1 day at room temperature	
20	Mean measured concentration (mg/mL)	19.03	18.97	Passed
	Percentage to the prepared concentration (%)	95.17	94.83	
	Coefficient of variation (%)	4.56	6.97	
	Residual ratio (%)	–	99.64	
60	Mean measured concentration (mg/mL)	58.00	–	
	Percentage to the prepared concentration (%)	96.66	–	
	Coefficient of variation (%)	4.82	–	
	Residual ratio (%)	–	–	
200	Mean measured concentration (mg/mL)	193.07	199.98	
	Percentage to the prepared concentration (%)	96.54	99.99	
	Coefficient of variation (%)	5.50	2.01	
	Residual ratio (%)	–	103.58	

*Acceptance criteria.
Concentration: Percentage of the mean measured concentration to the nominal concentration is within 100.0 ± 10.0%.
Homogeneity: Coefficient of variation is not more than 10.0%.
Stability: Residual ratio (percentage of the mean measured concentration after storage to the mean measured concentration of the preparation day) is within 100.0 ± 10.0%.
Numbers of measured samples are 6 for each concentration and time point.
Concentrations were analyzed by UV–Vis Spectrophotometer UV-2450 (Shimadzu Corporation., Kyoto).