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Original

A 90-day Feeding Toxicity Study of L-Serine in Male and Female Fischer 344 Rats

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Abstract: A subchronic feeding study of L-serine (L-Ser) was conducted with groups of 10 male and 10 female Fischer 344 rats fed a powder diet containing 0, 0.06, 0.5, 1.5 or 5.0% concentrations of L-Ser for 90 days. There were no toxicologically significant, treatment-related changes with regards to body weight, food intake, water intake or urinalysis data. In several of the hematology, serum biochemistry and organ weight parameters, significant changes were observed between some of the treated groups and the controls. All these changes, however, were subtle and lacked any corresponding pathological findings. In addition, the increased or decreased values remained within the range of the historical control values. In fact, histopathological assessment revealed only sporadic and/or spontaneous lesions. In conclusion, the no-observed-adverse-effect-level (NOAEL) for L-Ser was, therefore, determined to be at least a dietary dose of 5.0% (2765.0 mg/kg body weight/day for males and 2905.1 mg/kg body weight/day for females) under the present experimental conditions. (J Toxicol Pathol 2010; **23**: 39–47)

Key words: L-serine, toxicity study, Fischer 344 rat, feed

Introduction

L-Serine ((S)-2-amino-3-hydroxypropanoic acid; L-Ser) [CAS No.56-45-1] is a nutritionally non-essential amino acid that is synthesized from D-3-phosphoglycerate, an intermediate in the glycolytic pathway, or from glycine via the freely reversible serine hydroxymethyltransferase reaction. L-Ser is approved in Japan as an existing food additive for seasoning of the diet[1] and has recently become widely used as an ingredient of supplements, health foods and cosmetics. In addition, L-Ser and its metabolic byproducts have been recognized to be not only essential for cell proliferation, but also necessary for specific functions in the central nervous system[2,3]. Under these circumstances, potential risks of L-Ser must be well assessed and appropriately managed. There are, however, few reports available regarding the toxicity of L-Ser.

The oral 50% lethal dose (LD_{50}) of L-Ser for rats has been determined to be 14 (12.8–15.3) g/kg body weight[4]. In the reviews of Harper *et al*. in regard to the effects of disproportionate intake levels of amino acids[5] and of Garlick in regard to the safety of individual amino acids taken in excess relative to the amounts absorbed from dietary protein[6], excessive dietary levels of L-Ser have clearly been shown to depress the growth of rats fed low-protein diets. D-serine and DL-serine administered by several routes have been shown to affect growth and to produce renal necrosis[5,7]. On the other hand, L-Ser has been shown to induce significant increase of sister-chromatid exchanges in peripheral blood lymphocytes[8]. The information available concerning the potential risks of L-Ser is thus limited, and considering the human exposure situation, the risks need to be assessed in an urgent but careful manner by well-established protocols.

As previously described in our reports concerning safety assessment for L-aspartic acid[9], the Ministry of Health, Labour and Welfare (MHLW) of Japan is responsible for risk assessment and management of food additives in collaboration with the Food Safety Commission of Japan. In response to this issue, the MHLW has been funding research through grants, and the present 90-day feeding toxicity study of L-Ser in Fischer 344 rats was conducted as a part of this effort. It is important to note that the present study was conducted utilizing a purified 20% casein diet in order to exclude possible influence and interference due to the presence of other amino acids.

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Table 1. Composition of a Modified AIN-93G Diet Containing L-Ser

Ingredient	(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-Ser	X

Materials and Methods

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 the MHLW (Eika No. 29, March 22nd, 1996). The experimental protocol was approved prior to its execution by our in-house committee, which monitored every step during the experimentation for its scientific and ethical propriety, with strict obedience to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, Japanese Government Animal Protection and Management Law, Japanese Government Notification on Feeding and Safekeeping of Animals and Guidelines for Animal Experimentation (released by the Japanese Association for Laboratory Animal Science)[10].

Test chemical and diet preparation

L-Ser (lot number, 0000033701; purity, 100.3%, assessed 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 produce concentrations of 0 (control), 0.06, 0.5, 1.5 or 5.0% every 4 weeks; the diet composition is shown in Table 1. It should be noted here that the 0.06% group was used to assess the effects of a human-relevant dose (Worldsuppli.jp[2]) and thus should be considered as an additional group to a standard 3-dose safety assessment study protocol. The 0.06% dose is thus 8.3-fold lower than the immediate upper dose of 0.5%, while the other doses were set with the common ratios of 3.0–3.3. The L-Ser content in all experimental diets was analyzed at their preparation, and the actual values were 0.64 ± 0.02, 4.88 ± 0.22, 15.06 ± 0.06 and 54.08 ± 1.97 g/kg diet for the 0.06, 0.5, 1.5 and 5.0% doses, respectively. After keeping the 0.06 and 5.0% diets for 7 days at 22–24°C or for 30 days at 4°C, the contents of L-Ser were found to remain fairly stable, with values of 0.66 and 54.37 g/kg diet or 0.66 and 54.73 g/kg diet, respectively.

Animals and treatments

A total 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 to the control diet for 1 week before the start of the experiment. The rats were housed individually in stainless steel cages; kept under the controlled conditions of temperature (23 ± 2°C), relative humidity (55 ± 10%) 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 the acclimation and experimentation periods. After confirming normal health status at the end of the acclimation period, 50 rats of each sex were selected for use (5 rats of each sex being omitted), randomly allocated to 5 groups each consisting of 10 rats and given the control or experimental diets for 90 days. During the experimentation period, the rats were observed daily, and clinical signs and mortality (if any) were recorded. Body weight and food and water intakes were monitored weekly.

Animal sacrifice and assessments

At the end of the experimentation period of 90 days, all rats were deprived of food (but not water) overnight, and fresh urine samples were obtained for urinalysis of urobilinogen, occult blood, bilirubin, ketone, glucose, protein, pH and nitrous acid using reagent strips (N-Multistix, Siemens Medical Solutions Diagnostics Ltd., Tokyo, Japan). All rats were then lightly anesthetized and sacrificed by exsanguination after collecting blood samples via the abdominal aorta. Using the blood samples and subsequently prepared sera, hematological and serological examinations were performed. Hematological examination was carried out using an automatic analyzer (Sysmex KX-21NV; Sysmex Co., Hyogo, Japan) for the 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 a light microscopic observation of smeared specimens stained with a routine May-Grünwald-Giemsa protocol. Serum biochemistry determination was performed with another automatic analyzer (TBA-120FR; Toshiba Medical Systems Co., Tokyo, Japan) for the 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 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 glands, 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 vesicles, prostate, preputials, oviducts, vagina, lymph nodes (submandibular and mesenteric), thoracic aorta, sciatic nerve, spinal cords (cervical, mid-thoracic and lumbar), bones (femur and sternum) with bone marrows, Zymbal's glands and all gross lesions of each animal were fixed in 10% neutrally buffered formalin. Paraffin-embedded sections were then routinely prepared. All organs of the control and all treated groups were histopathologically examined by using these sections stained via a routine hematoxylin and eosin (HE) protocol.

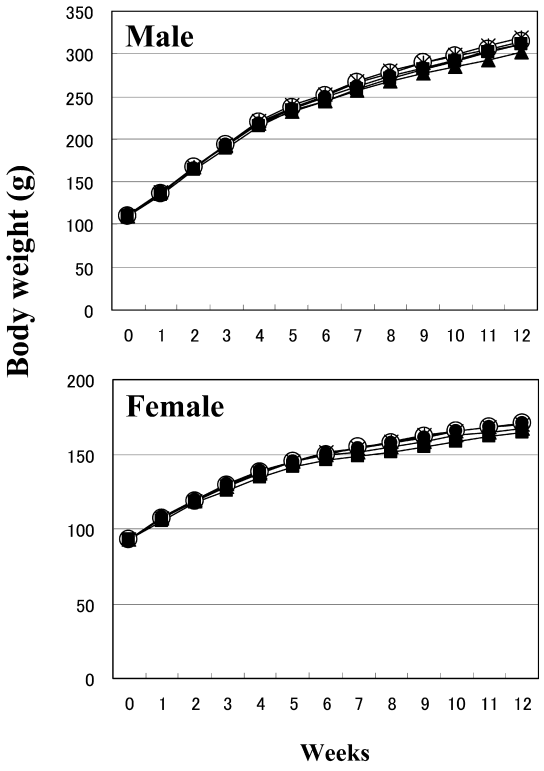

Fig. 1. Weekly changes in the average body weights of Fischer 344 rats (10 animals for each group) given L-serine at dietary doses of 0 (control, open circles), 0.06 (closed triangles), 0.5 (closed circles), 1.5 (asterisks) or 5.0% (closed squares) for 90 days.

Statistical analysis

For numerical data such as body and organ weights as well as hematological and serological outcomes, equality of means between the control and each treated group values was assessed by Bartlett's test. Homogeneity of variance was then analyzed by a one-way analysis of variance, and differences between the control and treated group values were evaluated by Dunnett's test. On the other hand, if the Bartlett's test was significant, the data were subjected to the Kruskal-Wallis test and the Dunnett's type rank sum test. For contingent data such as incidences of histopathological lesions and positive cases of urinalysis, differences between the control and treated group values were evaluated by the Fisher's exact probability test[11]. Statistical processing was conducted using a StatLight software (Yukms Ltd., Tokyo, Japan). Intergroup differences were considered statistically significant when *p*-values less than 0.05 were obtained.

Results

General findings

No rats died or became moribund until the end of the experiment. No treatment-related clinical signs were observed in the control or treated rats during the study. There were no significant differences in average body weights (Fig. 1) or average daily food and water intakes (Table 2) between the rats in the control group and those in any of the treated groups during the study (for both sexes). The average L-Ser intake of each group was calculated as demonstrated in Table 2.

Table 2. Average Daily Food, Water and Chemical Intakes in Fischer 344 Rats Given L-Ser for 90 Days

Item	Dietary dose of L-Ser (%)				
	0 (control)	0.06	0.5	1.5	5.0
Male					
Effective number of rats	10	10	10	10	10
Food intake (g/kg body weight/day)	55.3 ± 17.0[a]	55.2 ± 17.3	54.9 ± 16.8	55.2 ± 16.1	55.3 ± 15.7
Water intake (mL/kg body weight/day)	59.5 ± 17.4	59.4 ± 16.8	59.5 ± 16.7	63.4 ± 17.3	61.7 ± 16.1
Chemical intake (mg/kg body weight/day)	–	33.1 ± 10.4	274.7 ± 83.9	827.6 ± 241.7	2765.0 ± 785.3
Female					
Effective number of rats	10	10	10	10	10
Food intake (g/kg body weight/day)	59.4 ± 14.4	58.7 ± 13.5	58.5 ± 13.5	59.5 ± 13.1	58.1 ± 12.2
Water intake (mL/kg body weight/day)	80.1 ± 18.7	80.2 ± 18.8	80.2 ± 17.2	78.6 ± 18.0	74.9 ± 15.4
Chemical intake (mg/kg body weight/day)	–	35.2 ± 8.1	292.5 ± 67.5	892.6 ± 196.9	2905.1 ± 612.2

[a]Values are means ± standard deviations.

Table 3. Hematology in Fischer 344 Rats Given L-Ser for 90 Days

Item	Dietary dose of L-Ser (%)				
	0 (control)	0.06	0.5	1.5	5.0
Males					
Effective number of rats	10[a]	10	10	10	10
RBC ($\times 10^4/\mu$L)	903.9 ± 26.1[b]	910.7 ± 16.6	894.4 ± 15.5	896.6 ± 14.8	916.5 ± 16.0
HGB (g/dL)	15.1 ± 0.4	15.1 ± 0.3	14.9 ± 0.2	14.9 ± 0.1	15.0 ± 0.3
HCT (%)	47.4 ± 1.3	48.1 ± 1.1	47.1 ± 0.7	47.2 ± 0.7	47.6 ± 1.0
MCV (fL)	52.4 ± 0.3	52.8 ± 0.5	52.7 ± 0.2	52.6 ± 0.4	51.9 ± 0.3*
MCH (pg)	16.7 ± 0.2	16.6 ± 0.1	16.7 ± 0.1	16.6 ± 0.2	16.3 ± 0.2*
MCHC (g/dL)	31.8 ± 0.3	31.5 ± 0.2*	31.7 ± 0.2	31.6 ± 0.3*	31.4 ± 0.2*
WBC ($\times 10^2/\mu$L)	44.0 ± 10.0	46.0 ± 7.6	39.6 ± 9.7	44.7 ± 17.4	46.9 ± 16.4
Lymphocyte ($\times 10^2/\mu$L)	30.3 ± 7.6	31.7 ± 7.1	27.4 ± 9.1	29.6 ± 12.9	28.5 ± 9.4
Neutrophil ($\times 10^2/\mu$L)	12.8 ± 3.6	13.3 ± 3.1	11.3 ± 2.2	14.2 ± 5.8	17.2 ± 7.5
Eosinophil ($\times 10^2/\mu$L)	0.3 ± 0.3	0.3 ± 0.3	0.6 ± 0.6	0.4 ± 0.3	0.6 ± 0.5
Basophil ($\times 10^2/\mu$L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte ($\times 10^2/\mu$L)	0.6 ± 0.4	0.7 ± 0.5	0.3 ± 0.4	0.6 ± 0.3	0.7 ± 0.4
Others ($\times 10^2/\mu$L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PLT ($\times 10^4/\mu$L)	60.9 ± 4.0	60.9 ± 3.8	59.3 ± 1.9	59.4 ± 2.9	54.1 ± 10.5
Females					
Effective number of rats	9	10	10	10	8
RBC ($\times 10^4/\mu$L)	883.3 ± 14.4	876.9 ± 22.2	879.8 ± 14.2	877.8 ± 27.0	921.6 ± 13.0*
HGB (g/dL)	15.7 ± 0.2	15.5 ± 0.4	15.6 ± 0.2	15.4 ± 0.4	15.7 ± 0.4
HCT (%)	47.9 ± 0.9	47.7 ± 1.2	48.0 ± 0.7	47.6 ± 1.4	49.1 ± 0.9
MCV (fL)	54.3 ± 0.3	54.4 ± 0.3	54.5 ± 0.3	54.2 ± 0.3	53.3 ± 0.4*
MCH (pg)	17.8 ± 0.1	17.7 ± 0.1	17.7 ± 0.2	17.6 ± 0.2*	17.1 ± 0.2*
MCHC (g/dL)	32.8 ± 0.3	32.5 ± 0.3	32.4 ± 0.3*	32.4 ± 0.3*	32.0 ± 0.2*
WBC ($\times 10^2/\mu$L)	31.1 ± 5.9	31.1 ± 7.1	28.6 ± 6.5	33.7 ± 9.4	35.1 ± 6.7
Lymphocyte $\times 10^2/\mu$L)	25.2 ± 4.7	23.3 ± 7.8	20.6 ± 5.7	25.6 ± 7.8	27.5 ± 7.1
Neutrophil ($\times 10^2/\mu$L)	5.3 ± 1.8	7.2 ± 3.1	7.4 ± 2.1	7.7 ± 2.7	7.1 ± 3.1
Eosinophil ($\times 10^2/\mu$L)	0.3 ± 0.1	0.2 ± 0.3	0.3 ± 0.4	0.4 ± 0.3	0.3 ± 0.3
Basophil ($\times 10^2/\mu$L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte ($\times 10^2/\mu$L)	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.2
Others ($\times 10^2/\mu$L)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PLT ($\times 10^4/\mu$L)	53.8 ± 14.5	61.2 ± 5.5	61.9 ± 4.7	59.4 ± 13.1	66.9 ± 3.6*

[a] Effective numbers smaller than 10 occurred due to failure in collection of samples. [b] Values are means ± standard deviations. *Significantly different from the corresponding control values ($p<0.05$, Dunnett's test).

Urinalysis

Comparison of the results of urinalysis between the treatment and control groups revealed no treatment-related changes in the analyzed parameters.

Hematology and serum biochemistry

In the male rats, the MCV and MCH values of the 5.0% groups and MCHC values of the 0.06, 1.5 and 5.0% groups were significantly lower than those of the control group (Table 3). In the female rats, the RBC and PLT of the 5.0% group were significantly higher than those of the control group, while the MCV of the 5.0% group, MCH of the 1.5 and 5.0% group, MCHC of the 0.5% or greater groups were significantly lowered. However, all of these changes were subtle. The morphological findings and differential counts of leukocytes showed no significant adverse effects in any of the treated groups.

Serum biochemistry demonstrated that the values for TP of the 5.0% males and females, ALB of the 5.0% males, UA of the 1.5% or greater males, AST of the 5.0% males and females, and K of the 0.5% or greater males were significantly, although slightly, lower than those of the controls (Table 4). GGT of the 5.0% females, Na of the 1.5% males and 0.5% or greater females and Cl of the 0.5% or greater females were significantly, although slightly, higher than those of the controls (Table 4).

Organ weights

There were no significant differences in final body weights between the rats in the control and treated groups (for both sexes). In the male rats, the relative testes weight of the 0.06% group and relative kidney weight of the 5.0% group were significantly, although slightly, higher than those of the controls (Table 5). In the female rats, the relative weights of the brain, spleen, liver and kidneys of the 5.0% group were significantly, although subtly, higher than those

Table 4. Serum Biochemistry in Fischer 344 Rats Given L-Ser for 90 Days

Item	Dietary dose of L-Ser (%)				
	0 (control)	0.06	0.5	1.5	5.0
Male					
Effective number of rats	10	10	10	10	10
TP (g/dL)	6.89 ± 0.19[a]	6.86 ± 0.13	6.81 ± 0.11	6.76 ± 0.19	6.62 ± 0.18*
ALB (g/dL)	4.46 ± 0.09	4.43 ± 0.06	4.41 ± 0.07	4.41 ± 0.10	4.33 ± 0.08*
A/G	1.84 ± 0.09	1.83 ± 0.08	1.84 ± 0.08	1.88 ± 0.08	1.90 ± 0.11
GLU (mg/dL)	134.6 ± 9.8	126.7 ± 8.7	135.0 ± 11.3	129.7 ± 11.1	138.2 ± 14.1
T-CHO (mg/dL)	70.7 ± 7.4	66.5 ± 8.0	70.4 ± 9.5	68.5 ± 7.3	65.8 ± 5.8
TG (mg/dL)	91.9 ± 27.6	78.9 ± 24.7	91.0 ± 43.5	76.3 ± 31.3	76.2 ± 27.4
T-BIL (mg/dL)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
BUN (mg/dL)	14.6 ± 1.5	14.8 ± 1.4	14.5 ± 1.5	14.8 ± 1.9	14.6 ± 1.1
CRE (mg/dL)	0.27 ± 0.02	0.27 ± 0.02	0.27 ± 0.02	0.28 ± 0.01	0.27 ± 0.02
UA (mg/dL)	1.34 ± 0.20	1.26 ± 0.22	1.16 ± 0.17	1.13 ± 0.18*	1.07 ± 0.12*
AST (U/L)	91.2 ± 17.9	87.2 ± 9.2	87.1 ± 10.4	84.3 ± 8.8	74.0 ± 7.9*
ALT (U/L)	44.1 ± 4.6	41.6 ± 5.6	43.1 ± 6.6	43.8 ± 4.5	38.8 ± 6.7
GGT (U/L)	0.00 ± 0.00	0.30 ± 0.48	0.10 ± 0.32	0.20 ± 0.42	0.10 ± 0.32
ALP (U/L)	361.2 ± 26.3	364.2 ± 23.7	370.5 ± 22.7	356.8 ± 23.4	355.1 ± 27.9
Na (mmol/L)	158.5 ± 0.2	158.6 ± 0.1	158.6 ± 0.1	158.7 ± 0.2*	158.7 ± 0.1
K (mmol/L)	7.00 ± 0.13	6.89 ± 0.07	6.76 ± 0.10*	6.77 ± 0.10*	6.70 ± 0.11*
Cl (mmol/L)	117.8 ± 0.3	117.9 ± 0.2	117.9 ± 0.2	117.9 ± 0.3	117.9 ± 0.1
Ca (mg/dL)	10.8 ± 0.2	10.7 ± 0.1	10.7 ± 0.1	10.7 ± 0.2	10.7 ± 0.2
Female					
Effective number of rats	10	10	10	10	10
TP (g/dL)	6.68 ± 0.19	6.59 ± 0.16	6.64 ± 0.17	6.49 ± 0.23	6.43 ± 0.21*
ALB (g/dL)	4.40 ± 0.11	4.36 ± 0.09	4.38 ± 0.07	4.30 ± 0.13	4.28 ± 0.14
A/G	1.93 ± 0.08	1.95 ± 0.09	1.94 ± 0.12	1.96 ± 0.07	2.00 ± 0.08
GLU (mg/dL)	111.2 ± 26.9	106.8 ± 16.5	106.7 ± 11.8	99.1 ± 13.2	94.8 ± 6.6
T-CHO (mg/dL)	68.3 ± 6.0	66.9 ± 6.9	66.3 ± 6.9	64.2 ± 6.7	65.9 ± 4.2
TG (mg/dL)	17.3 ± 6.3	15.9 ± 6.7	14.0 ± 4.3	13.0 ± 3.5	13.4 ± 2.1
T-BIL (mg/dL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
BUN (mg/dL)	16.7 ± 1.4	17.7 ± 1.2	16.9 ± 2.6	16.7 ± 1.5	16.2 ± 2.1
CRE (mg/dL)	0.31 ± 0.04	0.31 ± 0.03	0.30 ± 0.01	0.30 ± 0.03	0.29 ± 0.01
UA (mg/dL)	1.36 ± 0.22	1.41 ± 0.20	1.38 ± 0.27	1.20 ± 0.16	1.19 ± 0.16
AST (U/L)	84.7 ± 10.7	101.2 ± 37.6	101.2 ± 46.2	113.3 ± 72.1	71.5 ± 4.9*
ALT (U/L)	32.2 ± 2.8	34.4 ± 6.4	31.9 ± 6.6	34.6 ± 11.1	30.6 ± 3.8
GGT (U/L)	0.20 ± 0.42	0.50 ± 0.53	0.40 ± 0.52	0.40 ± 0.52	0.88 ± 0.35*
ALP (U/L)	277.9 ± 35.5	279.8 ± 12.7	290.4 ± 32.9	289.7 ± 22.2	274.4 ± 18.6
Na (mmol/L)	158.6 ± 0.1	158.6 ± 0.1	158.8 ± 0.1*	158.8 ± 0.1*	158.9 ± 0.1*
K (mmol/L)	6.89 ± 0.10	6.80 ± 0.19	6.76 ± 0.21	6.71 ± 0.18	6.76 ± 0.14
Cl (mmol/L)	118.3 ± 0.2	118.4 ± 0.2	118.6 ± 0.2*	118.5 ± 0.3*	118.6 ± 0.2*
Ca (mg/dL)	10.4 ± 0.2	10.3 ± 0.2	10.4 ± 0.3	10.5 ± 0.4	10.4 ± 0.3

[a] Values are means ± standard deviations. *Significantly different from the corresponding control values ($p<0.05$, Dunnett's test).

of the controls (Table 5).

Pathology

No treatment-related macroscopic changes were observed in any organs of either sex. In the histopathological study, there were no treatment-related changes in any organs (for both sexes), whereas only sporadic spontaneous lesions were observed similarly in the control and treated animals. Spontaneous lesions in the liver and kidney are shown in Table 6.

Discussion

In the present 90-day feeding toxicity study of L-Ser in Fischer 344 rats, no treatment-related deaths, body weight changes, food and water intake changes or clinical signs were observed in either sex. In the literature, only a few inconsistent data are available with this regard. According to Muramatsu *et al.*, L-Ser at a dietary dose of 5.0% depressed the growth of Donryu rats during an experimentation period of 3 weeks, and the body weight gain was 34 ± 5 g and 69 ± 5 g for the L-Ser-treated and control animals, respectively[12]. Moreover, L-Ser at the levels of 2.1, 4.2 or 6.3% in diets

Table 5. Absolute and Relative Organ Weights in Fischer 344 Rats Given L-Ser for 90 Days

Item	Dietary dose of L-Ser (%)				
	0 (control)	0.06	0.15	1.5	5.0
Male					
Effective number of rats	10	10	10	10	10
Final body weight (g)	308.1 ±17.2[b]	294.0 ± 9.7	306.3 ± 14.8	313.4 ± 9.2	303.7 ± 15.8
Absolute organ weight (mg)					
Brain	2029.3 ± 34.6	2008.3 ± 44.0	2003.9 ± 14.0	2020.2 ± 24.5	1995.3 ± 19.8
Thyroids[a]	15.1 ± 2.4	12.7 ± 1.8*	14.9 ± 0.8	14.6 ± 1.6	15.2 ± 1.0
Heart	835.7 ± 35.4	835.5 ± 37.4	850.3 ± 55.8	855.1 ± 43.6	845.4 ± 59.5
Spleen	620.5 ± 64.2	588.5 ± 36.4	603.9 ± 39.8	618.6 ± 21.3	603.1 ± 33.4
Liver	6954.4 ± 525.3	6562.5 ± 353.5	6930.0 ± 608.6	6919.2 ± 467.1	6780.3 ± 560.2
Adrenals	39.52 ± 3.8	39.3 ± 2.2	37.6 ± 2.7	38.7 ± 2.7	38.1 ± 3.6
Kidneys	1752.9 ± 90.8	1705.6 ± 76.8	1739.5 ± 66.4	1792.6 ± 39.5	1782.3 ± 74.9
Testes	3061.7 ± 115.6	3130.7 ± 111.9	3044.9 ± 89.2	3111.9 ± 82.1	3037.7 ± 118.7
Relative organ weight (mg/100 g body weight)					
Brain	660.1 ± 30.2	683.5 ± 19.4	655.6 ± 32.1	645.2 ± 22.2	658.4 ± 32.7
Thyroids	4.92 ± 0.75	4.31 ± 0.63	4.88 ± 0.38	4.64 ± 0.43	5.01 ± 0.24
Heart	271.6 ± 10.1	284.3 ± 11.2	277.7 ± 15.2	272.9 ± 11.2	278.2 ± 10.5
Spleen	201.1 ± 11.9	200.1 ± 9.8	197.1 ± 7.4	197.5 ± 5.7	198.5 ± 3.2
Liver	2255.6 ± 67.6	2231.0 ± 60.1	2259.0 ± 102.6	2207.2 ± 113.4	2230.2 ± 96.7
Adrenals	12.9 ± 1.4	13.4 ± 0.8	12.3 ± 1.1	12.3 ± 0.8	12.5 ± 1.0
Kidneys	569.2 ± 15.4	580.0 ± 15.9	568.3 ± 12.2	572.2 ± 8.3	587.4 ± 23.5*
Testes	995.2 ± 40.8	1065.0 ± 27.0*	995.1 ± 25.3	993.4 ± 22.6	1001.7 ± 48.5
Female					
Effective number of rats	10	10	10	10	10
Final body weight (g)	163.5 ± 9.6	159.6 ± 9.9	163.1 ± 7.8	163.7 ± 7.6	154.3 ± 8.6
Absolute organ weight (mg)					
Brain	1821.1 ± 36.9	1851.4 ± 29.9	1852.6 ± 30.4	1853.8 ± 18.7	1845.6 ± 43.9
Thyroids	11.1 ± 1.3	11.0 ± 1.1	10.3 ± 1.3	13.1 ± 2.3*	10.8 ± 1.5
Heart	519.4 ± 26.1	517.6 ± 33.3	509.8 ± 28.4	519.1 ± 21.0	513.0 ± 28.9
Spleen	355.4 ± 12.0	360.5 ± 26.3	356.5 ± 23.4	377.1 ± 29.8	377.1 ± 29.8
Liver	3421.2 ± 221.5	3331.3 ± 237.1	3419.7 ± 185.7	3395.0 ± 226.1	3442.1 ± 204.1
Adrenals	42.3 ± 3.5	41.8 ± 3.4	42.9 ± 3.0	42.4 ± 1.8	42.3 ± 2.3
Kidneys	970.6 ± 38.5	966.2 ± 50.1	991.4 ± 38.5	1006.7 ± 44.7	1015.4 ± 59.9
Ovaries	48.2 ± 5.5	45.8 ± 4.8	47.2 ± 2.6	51.2 ± 6.5	46.5 ± 12.9
Uterus	382.8 ± 62.8	380.5 ± 72.2	415.5 ± 39.4	377.9 ± 77.4	342.1 ± 46.5
Relative organ weight (mg/100 g body weight)					
Brain	1117.5 ± 67.0	1163.9 ± 64.6	1138.2 ± 52.2	1134.4 ± 51.3	1192.4 ± 71.2*
Thyroids	6.85 ± 1.18	6.87 ± 0.64	6.30 ± 0.73	8.03 ± 1.49	6.91 ± 1.03
Heart	318.3 ± 16.6	324.7 ± 15.3	312.8 ± 16.0	317.3 ± 11.3	329.6 ± 8.2
Spleen	217.9 ± 12.4	226.0 ± 11.0	218.8 ± 13.5	230.2 ± 12.7	232.1 ± 9.7*
Liver	2094.3 ± 94.4	2090.5 ± 133.8	2097.5 ± 73.5	2072.2 ± 61.8	2221.4 ± 104.0*
Adrenals	26.0 ± 2.8	26.2 ± 2.3	26.3 ± 2.0	25.9 ± 1.4	27.4 ± 1.5
Kidneys	595.4 ± 39.4	606.6 ± 31.8	608.8 ± 27.3	615.2 ± 16.5	652.8 ± 32.8*
Ovaries	29.5 ± 3.0	28.7 ± 2.8	29.0 ± 2.3	31.3 ± 3.5	29.8 ± 8.1
Uterus	235.2 ± 42.8	238.7 ± 42.4	255.2 ± 26.5	230.6 ± 44.2	222.7 ± 27.1

[a] Thyroids were weighed after fixation. [b] Values are means ± standard deviations. *Significantly different from the corresponding control values (p<0.05, Dunnett's test).

resulted in growth reductions of 6, 25 and 29%, respectively[13].

In the L-Ser-administered groups, statistically significant changes were detected in some hematological parameters, such as RBC (high in the 5% females), MCV, MCH and MCHC (low in the 1.5 or 5% rats of both sexes). However, these hematological changes were slight and lacked dose-dependence within the range of the historic control values[14]. Furthermore, no abnormalities were observed in the microscopically assessed red blood cell figures in blood smears or on histopathological examination of hematopoietic organs. The observed hematological

Table 6. Histopathology in Fischer 344 Rats Given L-Ser for 90 Days

Item	Dietary dose of L-Ser (%)				
	0 (control)	0.06	0.15	1.5	5.0
Male					
Effective number of rats	10	10	10	10	10
Liver					
Necrosis of hepatocytes	2[a]	0	1	0	2
Microgranulation	0	1	2	0	1
Inflammatory cell infiltration	10	9	9	8	10
Kidney					
Regeneration of renal tubules	10	10	9	10	10
Inflammatory cell infiltration	0	0	1	0	1
Mineralization	3	2	4	4	4
Female					
Effective number of rats	10	10	10	10	10
Liver					
Necrosis of hepatocytes	2	2	0	0	1
Microgranulation	0	0	0	0	1
Inflammatory cell infiltration	10	10	10	9	8
Kidney					
Regeneration of renal tubules	5	3	1	1	2
Inflammatory cell infiltration	0	0	1	1	0
Mineralization	10	9	10	6	9

[a] Number of rats with lesions.

changes can therefore be considered incidental and not treatment-related.

The serum TP (in the 5.0% males and females) and ALB (in 5.0% males) levels were significantly lower than those of the control groups. Although these decreases showed dose-dependent tendencies, all changes were marginal, and the values in the L-Ser-administered groups were within the range of the historical control values[15]. Moreover, no corresponding pathological findings were observed. It is thus conceivable that the changes were toxicologically insignificant, even if they were treatment-related. Serine dehydratase (SDH) is induced by feeding of a synthetic diet containing L-Ser as a sole source of non-essential amino nitrogen (Serine diet). Of the non-essential amino acids tested, only L-Ser causes SDH induction, and the extent of such an effect depends on the serine content of the diet[16]. It has been suggested that SDH is induced as a response to the amount of surplus amino acids from dietary protein taken beyond the body's requirement[17,18]. According to the Institute of Life Science of the Ajinomoto Co., Inc., the supplier of the test compound, a lower blood threonine level was observed in rats administered L-Ser in diet for 14 days (Kawamata Y. *et al*., data not shown), and this lowered threonine level was considered to be related to induction of SDH (Sakai R., personal communication). If the lowered serum TP and ALB levels in the rats administered L-Ser (20% casein diet) in the present study were indeed treatment-related, these changes might be due to the decrease of threonine by induction of SDH as a sort of adaptive response.

In the literature, D-Ser is reported to be toxic and to cause kidney necrosis[6,8]. D-Ser administration has been shown to result in marked increases of urinary protein and amino acid content and glucose excretion, possibly by virtue of a direct effect on kidney function[19,20]. This D-Ser-induced aminoaciduria is attributed to impaired reabsorption of amino acids by injured proximal straight tubules, as well as to backward diffusion of amino acids from the interstitium[20]. Maekawa *et al*. determined that D-amino acid oxidase is involved in D-Ser-induced nephrotoxicity because intraperitoneally administered D-Ser causes glucosuria and polyuria in Fischer 344 rats but not in LEA/SENDAI rats lacking D-amino acid oxidase[21]. Regarding these renal toxicities of serine, Ganote *et al*. described that intraperitoneal administration of D-Ser to rats at a dose of 75 or 80 mg/100 g body weight causes reversible but selective diffuse necrosis of the proximal straight tubules but that similarly administered L-Ser does not exert this kind of effect[22]. In the present study, L-Ser produced no treatment-related functional or morphological renal injury in rats. It is thus likely that the renal toxicity of serine depends on its optical isomerism, namely, D-isomer-specific.

It is known that all of the assessed analogs and derivatives of L-Ser have sedative effects, although the amino acid acts in a different manner towards several behavioral stress markers for such things as spontaneous activity and distress vocalizations[23]. Asechi *et al*. has confirmed that L-Ser may be effective in improving anxiety or sleep disorders induced by psychological stressors. L-Ser administered intracerebroventricularly has sedative and

hypnotic effects on neonatal chicks under acute stressful conditions[24]. In the present study using young adult rats, no signs or symptoms suggesting neurotoxicity of L-Ser were observed.

Kaneko *et al*. recently reported the results of a subchronic toxicity study of L-Ser in Sprague-Dawley rats by gavage[25]. They noted no treatment-related, toxicologically significant changes in body weights, food consumption, outcomes of urinalysis, hematology or serum biochemistry, organ weights, or macroscopic or histopathological examinations in any treatment groups, and the no-observed-effect level (NOEL) was 3000 mg/kg body weight/day for both sexes[25]. In our present study using a purified 20% casein diet in Fischer 344 rats, several significant changes were observed between some of the treated groups and the controls in hematology, serum biochemistry and organ weights. However, these changes were subtle and lacked dose-dependence, and no abnormalities were observed that corresponded to pathological findings. Although there were consistent and inconsistent changes in several of the assessed parameters, the outcomes of both studies were thus fundamentally identical. Based on these 2 studies, therefore, it is safe to infer that L-Ser is not harmful in rats up to considerably high dose levels when exposed orally.

In conclusion, the no-observed-adverse-effect-level (NOAEL) for L-Ser was determined to be at least a dietary dose of 5.0% (2765.0 mg/kg body weight/day for males and 2905.1 mg/kg body weight/day for females) under the present experimental conditions.

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