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Tumorigenicity study of L-theanine administrated orally to mice

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Abstract

L-Theanine was orally administered at a concentration of 0–5% (maximum tolerated dose) of the diet to B6C3F$_1$ mice for 13 weeks in a subacute test and 78 consecutive weeks, in a chronic toxicity test. There were no significant differences in intake of diet, weight gains or survival rates between treated and control groups. Unignorable differences in tumor incidence were observed at the end of 78 weeks; however, total number of tumors decreased significantly in the L-theanine administered groups. Thus, the long-term oral administration of L-theanine to mice did not show any chronic toxicological evidence or tumorigenic aberration.

Keywords: Theanine; Amino acid; Tumorigenicity; Mice

1. Introduction

L-Theanine (L-glutamate-γ-ethylamide, $C_7H_{14}N_2O_3$, CH_3–CH_2–NH–CO–CH_2–CH_2–CH(NH_2)–COOH) is found in Gyokuro (the highest grade of green tea) as one of the amino acids responsible for the exotic taste of green tea (known as "umami") (Sakato, 1950). It was named after the former scientific name of green tea, *Thea sinensis*. L-Theanine is a unique amino acid as it is found only in the tea plant, with the exception of one type of mushroom and certain species of genus *Camellia*, *C. japonica* and *C. sasanqua*. L-Theanine constitutes between 1% and 2% of the dry weight of tea leaves (Mukai, Horie, & Goto, 1992). L-Theanine was registered with the Japanese food additives in 1964, and has been used to fortify flavours of lower grade green teas, including Sencha and Bancha. A large scale industrial production method for L-theanine as a food additive was established by Abelian et al. (1993). Since then, various kinds of experiments, both *in vitro* and *in vivo*, and human trials have been performed and L-theanine has been shown to have physiological activities, and has been used in various food applications (Juneja, Chu, & Okubo, 1999).

However, only a few reports on acute and subacute toxicity of L-theanine have been published and no chronic toxicity or tumorigenicity has been reported so far. Ishidate et al. (1984) have reported about safety of L-theanine in a series of trials, including mutagenecity test, an acute toxicity test and a subacute toxicity test. They revealed that L-theanine did not show any abnormality in Salmonella/microsome tests (Ames tests) or chromosomal aberration tests using culture cells of Chinese hamster. Juneja et al. (1999) indicated that the LD_{50} of L-theanine might be more than 5.00 g/kg BW.

In this study, tumogenecity and subacute and chronic toxicity were investigated in order to evaluate the safety of L-theanine.

2. Materials and methods

2.1. Subacute toxicity study

To estimate the maximum tolerated dose (MTD) to be used in a chronic toxicity study, several concentrations of L-theanine were used for a subacute toxicity study of 13 weeks in mice.

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Fifty B6C3F1 mice of each sex were divided into five groups, respectively. Based on the result of a preliminary test, 5% (for a maximum dosage in diet), 2.5%, 1.25% and 0.6% of L-theanine were administered to each test group for each sex. One group for each sex was set for the control group (0%). The mice (of each sex) were housed in plastic cages and kept in an air-conditioned room.

Test substance, L-theanine (Ajinomoto Co., Ltd.), was premixed in basal diet (CRF-1) prepared by Oriental Yeast Co., Ltd. (Shizuoka, Japan) according to the above dosages. All mice were given diet pellets *ad lib*. for 13 consecutive weeks. The mice in the control group were given basal diet and tap water for the test period.

Total amounts of L-theanine during the test period were calculated. The mice were weighed every week after starting the administration and mean body weight in each group was calculated. Amounts of diet consumed in each group were measured weekly from the first week after the start of administration.

At the end of the 13 week test periods, all animals were killed by ether and autopsied. Organs and tissues, including heart, lung, submaxillary gland, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, ovary, thyroid gland, adrenal gland, thymus, spleen, bone marrow, brain (cerebrum, cerebellum) and spinal cord were observed for histological aberration judgment.

2.2. Chronic toxicity study

One hundred and fifty B6C3F1 mice of each sex were randomly divided, into three groups composed of 50 animals in each group. Based on the MTD in the above subacute toxicity study, 5%, 2.5% and 0% of L-theanine were given to each group. Five animals in each sex and group were housed in a plastic cage and kept in the air-conditioned room.

L-Theanine was premixed in basal diet (CRF-1) prepared by Oriental Yeast Co., Ltd. according to the above dosages. The mice were fed test diets *ad lib.* for 78 consecutive weeks. The mice in the control group were given basal diet and tap water for the same period.

The mice were weighed every week during the first 4 weeks after starting administration and every 4 weeks thereafter. Diet consumption in each group was also measured according to the same schedule as for measurement of body weight.

The mice surviving to the end of the 78 week experimental period were killed by ether and autopsied for pathological study.

3. Results

3.1. Subacute toxicity study

The average values of L-theanine intake per mouse for 13 weeks obtained by calculation from amounts of fed diet were, 19.8 g (5% group), 10 g (2.5% group), 5 g (1.25% group) and 2.6 g (0.6% group) for male mice, and 16.4 g (5% group), 8.4 g (2.5% group), 4.2 g (1.25% group) and 2.1 g (0.6% group) for female mice. The final body weights after 13 weeks were, 35.8 g (5% group), 38.2 g (2.5% group), 37.5 g (1.25% group), 38.0 g (0.6% group) and 36.2 g (0% group) for male mice, and 26.7 g (5% group), 27.5 g (2.5% group), 29.0 (1.25% group), 24.2 g (0.6% group) and 24.5 g (0% group) for female mice. From the above results, total amounts of diets and weight gains showed slight differences between male and female groups, however, there were no statistical differences among test groups.

No mortality among sex and groups was observed during the test period. No particular pathological changes, including degeneration, atrophy, necrosis, inflammation or tumor were noted in any organs or tissues by pathological examination. There were no histological abnormalities in any organ observed in this trial.

From these results, the MTD of L-theanine was estimated to be more than 5%.

3.2. Chronic toxicity study

The average diet consumption per day, final body weight and amount of daily intake of L-theanine estimated, based on the mean diet consumption, are shown in Table 1. Slight differences, both in diet consumption and L-theanine consumption between male and female mice were observed, but no statistical differences were found among test groups within each sex.

Changes in mean body weights in each group during the test period are shown in Fig. 1. Mean body weight after the 20th week tended to be slightly lower in the 5% and 2.5% groups in comparison with that in the control group. However, there were no statistically significant differences.

Changes in survival rates in test groups in male and female B6C3F1 mice during the administration period are shown in Fig. 2. Small numbers of male mice with tumors were observed from 40 weeks after the start. On the other hand, about 95% of female mice survived during the test period.

Numbers of surviving mice and numbers of mice bearing malignant tumors are shown in Table 2. Numbers of tumor-bearing mice in test groups tended to be lower than in control groups (both male and female). Statistical significance was observed in test groups of male mice.

Table 1
Consumption of L-theanine by mice given 2.5% or 5.0% in the diet for 78 weeks

Sex	L-Theanine conc (% in diet)	Diet consumed mg/mouse/day (mg/kg)	Final body weight (g)	L-Theanine consumed mg/mouse/day (mg/kg)
Male	0 (control)	4.8	50.6 ± 5.2	0
	2.5	4.1	54.5 ± 5.3	0.113
	5	4.5	49.7 ± 6.8	0.225
Female	0 (control)	4.4	59.0 ± 7.5	0
	2.5	4.2	55.0 ± 6.9	0.105
	5	4.3	52.2 ± 6.7	0.215

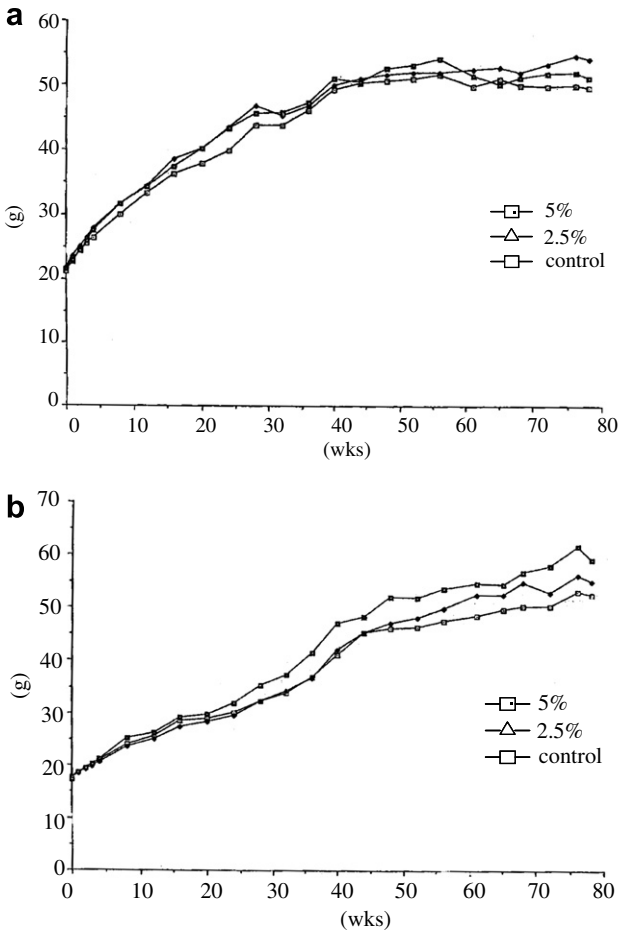

Fig. 1. Average body weights of male (a) or female (b) mice of the 0% group (■), 2.5% group (▲) and 5% group (□).

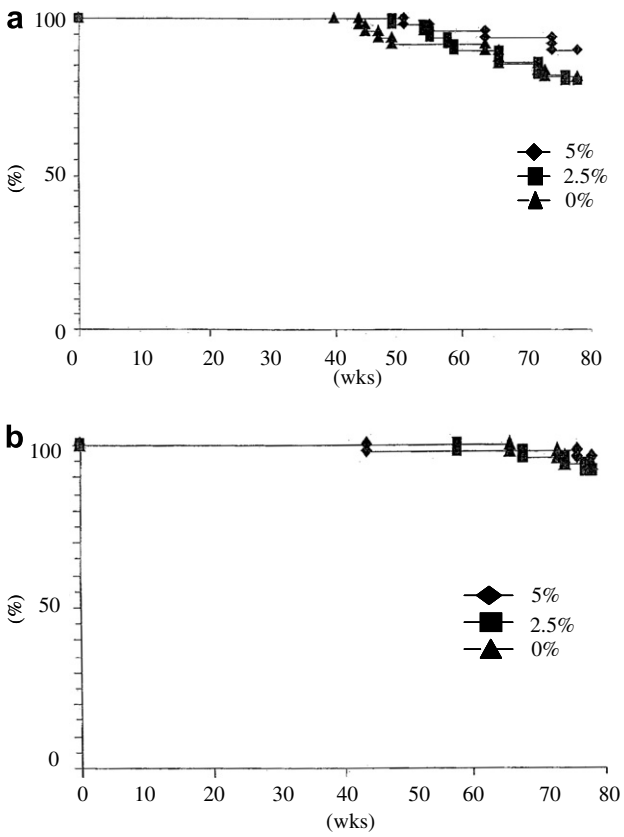

Fig. 2. Percent survival of male (a) or female (b) mice of the 0% group (▲), 2.5% group (■) and 5% group (♦).

Histological types of neoplasm in organs and incidence of tumors in test groups are shown in Table 3. Several kinds of neoplasm were observed, broadly in test and control groups of both sexes. Relatively high incidences were observed for bronchiolo-alveolar adenoma in lung, hepatocellular adenoma and hemangioma in liver in the control group of male mice. The other types of tumors were too infrequent to find statistical difference. The total number of tumors in female mice was lower than that in the male groups. It is noteworthy that total number of tumors decreased in a dose-dependent manner in both sexes, that is, as the concentration of L-theanine increased, the numbers of tumors decreased.

4. Discussion

Based on former research by Juneja et al. (1999), a concentration of 5% in the test diet was investigated as the maximum dose in this subacute toxicity study. In this study, there were no differences in weight gains, mortality, pathological changes among test groups or between male and female mice groups. These results indicated that the TMD of L-theanine might be more than 5%.

Table 2
Incidence of tumors in mice given L-theanine in the diet for 78 weeks

Sex	L-Theanine conc (% in diet)	No. of 'effective' mice	Mean survival time (week) in 'effective' mice	No. of tumor-bearing mice (% of 'effective' mice)
Male	0 (control)	43	76.9 ± 5.2	25 (58.1)
	2.5	41	77.7 ± 5.3	10 (24.3[b])
	5	45	78	9 (20[a])
Female	0 (control)	47	78	8 (17)
	2.5	46	78	5 (10.9)
	5	48	78	4 (8.3)

'Effective' mice are those that survived beyond week 78, during which the first animal in the study died with a tumor.
a and b significantly different from 0% group at $p < 0.0002$ and $p < 0.0017$, respectively.

In the chronic toxicity study (78 weeks), there were slight differences for the mean body weight gains and survival rates; however, these differences were not statistically significant.

On the other hand, there were unignorable differences in total number of tumor-bearing mice. Total numbers of tumors in both sexes decreased in L-theanine intake groups, and statistically significant differences (dose-dependent manner) were observed in the male mice (Tables 2 and 3).

Zhang, Miura, and Yagasaki (2001) demonstrated that L-theanine directly suppressed invasion of cancer cells

Table 3
Histological distribution of benign and malignant tumours in mice given L-theanine

Organ	Histological type of neoplasm	Incidence (% of 'effective' mice)					
		Male mice			Female mice		
	L-Theanine conc (% in diet)	0	2.5	5	0	2.5	5
Lung	Bronchiolo-alveolar adenoma	7 (16.2)	2 (4.9)	2 (4.4)	2 (4.3)	1 (2.2)	1 (2.1)
	Bronchiolo-alveolar carcinoma	3 (7.0)	1 (2.4)	0	0	0	1 (2.1)
Liver	Foci of cellular alteration	3 (7.0)	3 (7.3)	1 (2.2)	0	0	0
	Hepatocellular adenoma	11 (25.6)	5 (12.2)	5(11.1)	3 (6.4)	0	0
	Hepatocellular carcinoma	1 (2.3)	1 (2.4)	1 (2.2)	0	0	0
	Peliosis	1 (2.3)	1 (2.4)	0	0	0	1 (2.1)
	Hemangioma	5 (11.6)	0	0	0	0	0
	Angiosarcoma	0	0	0	0	0	0
RES	Malignant lymphoma	0	1 (2.4)	0	1 (2.1)	0	0
Soft tissue	Fibrosarcoma	0	1 (2.4)	0	0	0	0
	Hemangioma	0	0	0	0	0	1 (2.1)
Skin	Angiosarcoma	1 (2.3)	0	0	0	0	0
	Sebaceous carcinoma	0	0	0	0	0	0
Spleen	Hemangioma	0	0	0	1 (2.1)	1 (2.2)	0
	Angiosarcoma	1 (2.3)	0	1 (2.2)	1 (2.1)	1 (2.2)	0
Heart	Hemangioma	1 (2.3)	0	0	0	0	0
Ovary	Serous cystadenoma	–	–	–	0	0	1 (2.1)
	Immature teratoma	–	–	–	0	1 (2.2)	0
	Total no. of tumours	34	15	10	8	4	5

in vitro and *ex vivo* and that the mechanisms of its action might be related to the *N*-methyl-D-aspartate (NMDA) receptor. Sadzuka, Sugiyama, and Suzuki (2001) suggested an enhancing effect of L-theanine on the antitumor activity of cisplatin or doxorubicin by an inhibition experiment of the glutamate transporter *in vitro*. These studies indicate that L-theanine may be useful for cancer chemotherapy as a substance against drug-sensitive, drug-resistant metastatic tumors. Further research is necessary to evaluate the inhibitory effect of L-theanine on carcinogenesis, using model animals, and to clarify exact mechanisms.

In conclusion, it is suggested that middle to long-term daily administration of L-theanine to B6C3F1 mice would cause no undesirable effects (not only physical changes such as intake of diets or weight gains, but also physiological traits such as survival rate or types of neoplasm in various organs).

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