Time-Dependent Effects of L-Tryptophan Administration on Urinary Excretion of L-Tryptophan Metabolites

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Summary We have previously reported that dietary supplementation with up to 5.0 g/d of L-tryptophan (L-Trp) for 21 d has no adverse effects, judging from the levels of general blood variables, in healthy women. We performed a randomized, double-blind, placebo-controlled, crossover intervention study in 17 apparently healthy Japanese women. The subjects were randomly assigned to receive a placebo (0 g/d) or 1.0, 2.0, 3.0, 4.0, or 5.0 g/d of L-Trp for 21 d each with a 5-wk washout period between trials. We examined the 24-h urine profiles on days –1 (1 d before starting L-Trp), 7, 14, and 21 to determine whether administration of L-Trp at doses of up to 5.0 g/d affects time-dependent urinary excretion of L-Trp or its metabolites in healthy women. The urinary excretion of L-Trp, kynurenine acid, 3-hydroxykynurenine, xanthurenic acid, 3-hydroxyanthranilic acid, quinolinic acid, N1-methylnicotinamide, N1-methyl-2-pyridone-5-carboxamide, and N1-methyl-4-pyridone-3-carboxamide increased in an L-Trp dose-dependent manner on day 7. The amount of urinary excretion of these compounds was unchanged on days 14 and 21. The urinary excretion of serotonin, 5-hydroxyindole-3-acetic acid, 2-oxoadipic acid, and nicotinamide was unaffected by L-Trp at any of the doses tested. L-Trp doses had weak effects on the urinary excretion of kynurenine and anthranilic acid. Based on these findings, we conclude that there are no time-dependent effects of L-Trp administration in urinary excretion of L-Trp metabolites. Additionally, L-Trp and its metabolites do not accumulate in the body.

Key Words L-tryptophan, kynurenine metabolite, serotonin, urine, human

We have previously reported that dietary supplementation with up to 5.0 g/d of L-tryptophan (L-Trp) has no adverse effects, such as altering general blood variables, in healthy women (1). In rats, administration of L-Trp causes an increase in Trp 2,3-dioxygenase (TDO) via substrate or cofactor enhancement, in which the degradation of the pre-existing apoenzyme is decreased despite a normal synthesis rate (2–5). Administration of hydrocortisone increases TDO activity, causing a reduction in plasma, liver, and brain concentrations of L-Trp. A change in brain L-Trp concentrations leads to a reduction in the synthesis of the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) (6, 7). These changes (2–7) are observed within several hours after starting administration of L-Trp and hydrocortisone. There are currently no data describing the effects of continuous administration of L-Trp on its metabolism and urinary excretion in vivo.

We performed a randomized, double-blind, placebo-controlled, crossover intervention study in 17 apparently healthy Japanese women in which the subjects were randomly assigned to receive a placebo (0 g/d) or 1.0, 2.0, 3.0, 4.0, or 5.0 g/d of L-Trp for 21 d each with a 5-wk washout period between trials (1). In our study, we collected 24-h urine samples on days –1 (1 d before starting L-Trp administration), 7, 14, and 21 of each treatment period to determine whether continuous administration of L-Trp for 21 d increases the urinary excretion of L-Trp and/or its metabolites. We describe the temporal changes in urinary excretion of L-Trp and its metabolites following dietary supplementation of L-Trp. The metabolic pathway of L-Trp is shown in Fig. 1.

MATERIALS AND METHODS

This study, which was conducted between September 2010 and October 2011, was reviewed and approved by the Ethical Committee of The University of Shiga Prefecture, and was conducted according to the ethical guidelines of the Declaration of Helsinki. All of the participants provided written informed consent.

Chemicals. Pharmaceutical grade L-Trp was provided by Ajinomoto Co., Inc. (Tokyo, Japan). Serotonin (5-HT) creatinine sulfate, nicotinamide (Nam), pyridoxal 5′-phosphate (PLP), riboflavin, anthranilic acid (AnA), 3-hydroxykynurenine (3-HK), quinolinic acid (QA), and 2-oxoadipic acid (2-OAA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-Pyridoxic acid (4-PIC), which was manufactured by ICN Pharmaceuticals (Costa Mesa, CA), was...
purchased from Wako Pure Chemical Industries. Kynurenine (Kyn) sulfate, xanthurenic acid (XA), kynurenic acid (KA), 3-hydroxyanthranilic acid (3-HA), and N$^1$-methylnicotinamide (MNA) chloride were obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). 5-Hydroxyindole-3-acetic acid (5-HIAA) was purchased from Sigma-Aldrich Chemicals (St. Louis, MO). N$^1$-Methyl-2-pyridone-5-carboxamide (2-Py) (8) and N$^1$-methyl-4-pyridone-3-carboxamide (4-Py) (9) were synthesized as previously described. All other chemicals were of the highest commercially available purity.

Subjects. Female Japanese university students were recruited from The University of Shiga Prefecture. The purpose and protocol of this study were explained to all of the participants, and written informed consent was obtained. Women diagnosed with cold or influenza, and those who had taken multivitamin supplements at least once during the previous month, were excluded from the study. All of the subjects passed regular medical examinations at the university. Of the 21 apparently healthy female Japanese students who participated in the study, 17 subjects (aged 18–26 y; mean $\pm$ standard deviation [SD]: 20.2 $\pm$ 0.6 y) completed the study.

Study design. In this randomized, double-blind, placebo-controlled, crossover intervention study, subjects were assigned to receive 0 (placebo; starch), 1.0, 2.0, 3.0, 4.0, or 5.0 g/d of l-Trp in a random order for 21 d each with a 5-wk washout period between trials. All of the subjects participated in all six trials, and received all of the test doses of l-Trp. l-Trp or the placebo was administered at each meal, three times per day, for 21 d. The l-Trp and placebo tablets were similar in appearance. Their compositions are reported in our previous report (1). Participants assigned to 0 g/d l-Trp (placebo) took 20 placebo tablets per meal, those assigned to 1.0 g/d l-Trp took four l-Trp tablets and 16 placebo tablets, and those assigned to 5.0 g of l-Trp took 20 l-Trp tablets. To monitor compliance, the participants were instructed to record their consumption of tablets at each meal, and to give these records to the investigators at each visit. Twenty-four-hour urine samples were collected on days –1 (1 d before starting administration of the test drug), 7, 14, and 21 in each trial. All of the trials started at 09:00 h on day 1 and ended at 09:00 h on day 22.

Analyses. l-Trp and its metabolites, including 5-HT, 5-HIAA, Kyn, KA, AnA, 3-HK, QA, 3-HA, 2-OAA, QA, MNA, Nam, 2-Py, and 4-Py, were measured as previously described (10). The urinary concentrations of free riboflavin (11) and 4-PIC (12), a catabolite of vitamin B$_6$, were also measured as previously described.

Statistical analyses. Two-way repeated analysis of
High-Dose l-Trp Administration and l-Trp Metabolism

Variance (ANOVA) was used to determine the significance of differences in mean metabolite concentrations among study days and dose groups, followed by Bonferroni’s post hoc test. When two-way repeated ANOVA indicated the presence of a study day-dose interaction, one-way ANOVA was conducted followed by Tukey’s multiple-comparison test among the groups. Values of $p < 0.05$ were considered statistically significant. Prism software version 5.0 (Graph Pad Software, San Diego, CA) was used for all statistical analyses.

RESULTS AND DISCUSSION

l-Trp and 5-HT

5-HT, a neurotransmitter, which also stimulates contraction of smooth muscle in the stomach and small intestine, is produced from l-Trp. Over 95% of 5-HT is produced in the digestive tract (13). Accordingly, urinary excretion of 5-HT and its metabolite, 5-HIAA, reflects systemic l-Trp metabolism via the 5-HT pathway. Figure 2A, B, and C show the temporal changes in urinary excretion of l-Trp, 5-HT, and 5-HIAA, respectively. The urinary excretion of l-Trp was higher on day 14 than on days 7 and 21 in the 5 g/d l-Trp administration group, but it was unchanged in the other groups on days 7 and 21. This finding suggests that administered l-Trp was metabolized and excreted in an unchanged form in the urine. By contrast, urinary excretion of 5-HT and 5-HIAA remained constant from days 21 to 21.

Metabolites of the Kyn pathway

Over 90% of l-Trp is metabolized by the Kyn pathway (14, 15). The metabolic fate of l-Trp in healthy women has been reported (10), and we previously reported its fate in healthy women who were administered up to 5.0 g/d l-Trp for 21 d (1). In the present study, we compared the urinary excretion profiles of l-Trp and its metabolites on days −1, 7, 14, and 21. The urinary excretion of the major metabolites in the
l-Trp→3-HA pathway, including Kyn, AnA, KA, 3-HK, XA, and 3-HA, is shown in Fig. 3. In Fig. A, B, D, E, and F, the main effects of study days and dose were found to be significant ($p=0.0008$ in Fig. 3A, $p<0.0001$ in Fig. 3B, $p<0.0001$ in Fig. 3D, $p<0.0001$ in Fig. 3E, and $p<0.0001$ in Fig. 3F). Therefore, one-way ANOVA was conducted, followed by Tukey’s multiple-comparison test among all of the groups. There were no significant differences in the amount of urinary excretion among the study days within the same dose group. Of these metabolites, urinary excretion was greatest for 3-HK on days 7, 14, and 21 in subjects administered 5.0 g/d l-Trp. In Fig. 3C, the main effects of study days and dose were not found ($p=0.9916$). Accumulation of an intermediate metabolite generally means that the capacity of the metabolic enzyme has reached its limit. Kynureninase, a PLP-dependent enzyme localized in the cytosol, catalyzes the reaction 3-HK→3-HA (16) and the reaction kynurenine→AnA. AnA is considered to be a side-product or end-product of the l-Trp→Kyn pathway, rather than an intermediate metabolite. In the current study, the urinary excretion of AnA was low, with an excretion rate of approximately 4 μmol/d after administration of 5.0 g/d l-Trp. By contrast, the urinary excretion of 3-HK was approximately 500 μmol/d at administration of 5.0 g/d l-Trp. These findings indicate that the preferred substrate of human kynureninase is 3-HK but not Kyn. Tanizawa et al. (17) reported that pig liver kynureninase prefers 3-HK as a substrate, while Lima et al. (18) reported that the constitutive kynureninases in humans and other eukaryotic species preferentially catalyze the hydrolytic cleavage of 3-HK to 3-HA and alanine.

A similar phenomenon was observed in the urinary excretion of 3-HA in our study. This finding is inconsistent with in vitro data because the metabolic enzyme, 3-HA 3,4-dioxygenase, has high activity in rats and mice. Generally, 3-HA 3,4-dioxygenase activity relative to liver tissue mass is approximately 1,000 times higher than that of the other enzymes involved in the l-Trp→Kyn pathway (19). Currently, however, we have no data on the activity of 3-HA 3,4-dioxygenase in humans.

**Metabolites of the glutarate and Nam pathways**

The unstable intermediate α-amino-β-carboxymuconate-ε-semialdehyde (ACMS) results from metabolism of 3-HA by 3-HA 3,4-dioxygenase. ACMS is mostly metabolized into α-aminomuconate-ε-semialdehyde (ACM) by ACM decarboxylase. ACM is then metabolized to acetyl-CoA via 2-OAA. This pathway is known as the glutarate pathway. Figure 4 shows the temporal change in urinary excretion of 2-OAA (Fig. 4A), QA (Fig. 4B), Nam (Fig. 4C), MNA (Fig. 4D), 2-Py (Fig. 4E), and 4-Py (Fig. 4F). The main effects of study days and dose were not found for 2-OAA and Nam ($p=0.9953$ and $p=0.9864$, respectively). The main effects of study days and dose were found to be significant for QA, MNA, 2-Py, and 4-Py (all $p<0.0001$). Therefore, one-way ANOVA was conducted, followed by Tukey’s multiple-comparison test among all of the groups. There was no significant difference in the amount of urinary excretion among the study days within the same dose group. The urinary excretion of 2-OAA was unaffected by the duration of l-Trp administration. Nam is low in humans, and was thus close to the lower limit of detection (9). Shibata et al. (20) reported that Japanese adult men did not eliminate detectable amounts of Nam, even when Nam was administered at a dose of 150 mg/d for approximately

![Fig. 4. Effects of continuous l-Trp administration for 21 d on the urinary excretion of 2-OAA (A), QA (B), Nam (C), MNA (D), 2-Py (E), and 4-Py (F). Each point represents the mean±SD for 17 subjects. ●: 0 g/d l-Trp (placebo), ○: 1.0 g/d l-Trp, ■: 2.0 g/d l-Trp, □: 3.0 g/d l-Trp, ▲: 4.0 g/d l-Trp, △: 5.0 g/d l-Trp.](image-url)
1 y. In the present study, urinary excretion of Nam was low, even at the highest dose of l-Trp. By contrast, other metabolites, including QA, MNA, 2-Py, and 4-Py, were extensively eliminated into urine. The urinary excretion levels of these metabolites were unaffected by the duration of administration.

Figure 5A shows the sum of the urinary excretion of Nam, MNA, 2-Py, and 4-Py. In Fig. 5A, the main effects of study days and dose were found to be significant (p<0.0001). Therefore, one-way ANOVA was conducted, followed by Tukey’s multiple-comparison test among all of the groups. There was no significant difference in the amount of urinary excretion among the study days within the same dose group. The sum urinary excretion did not change over time (Fig. 5A). Figure 5B shows the urine excretion ratio of (2-Py+4-Py)/MNA. In Fig. 5B, the main effects of study days and dose were not found (p=0.4839). This ratio remained constant from days −1 to 21.

Excretion of riboflavin and vitamin B₆

Riboflavin and vitamin B₆ are involved in the metabolism of l-Trp in a pathway that involves Kyn 3-monoxygenase, an FAD-dependent enzyme (21), and the PLP-dependent enzymes kynureninase, Kyn aminotransferase, and 5-hydroxytryptophan decarboxylase (16, 22, 23). l-Trp loading to vitamin B₆-deficient humans results in a marked increase in the urinary excretion of Kyn and XA (19, 24). Therefore, vitamin B₂- or vitamin B₆-deficiency could be related to the increased urinary excretion of l-Trp metabolites in the present study. Normally, the amount of urinary excretion of water-soluble vitamins reflects the vitamin’s nutritional status (25), particularly a surplus of the vitamin. Therefore, we measured the amount of urinary excretion of riboflavin and vitamin B₆ to determine whether any of our subjects were vitamin B₂- or vitamin B₆-deficient at the time of this study. Figure 6 shows the temporal changes in the urinary excretion rates of riboflavin (Fig. 6A) and 4-PIC (Fig. 6B), a catabolite of vitamin B₆. The main effects of study days and dose were not found for riboflavin and 4-PIC (p=0.5452 and p=0.7842, respectively). Therefore, the amount of urinary excretion of riboflavin and 4-PIC was unaffected by the duration of l-Trp administration. Based on these data, the vitamin B₂ and B₆ nutritional status of our subjects was good throughout the study. We previously reported that the dietary intakes of vitamins B₂ and B₆ were 1.2 mg/d and 0.8 mg/d, respectively (1). These values are consistent with the average daily intake of these vitamins in healthy young women (26), indicating that our subjects were not vitamin B₂- or vitamin B₆-deficient. Therefore, the present data indicated that increasing urinary excretion of l-Trp metabolites was not related to vitamin B₂ or vitamin B₆ deficiency, and additional dietary supplementation of these vitamins was not necessary, even when the subjects were administered 5.0 g/d l-Trp for 21 d.

Limitations

There are some limitations to the present study. We did not collect urine samples from days 1 to 6, which prevented us from precisely determining when the urinary excretion of l-Trp and its metabolites started to increase. Therefore, we cannot exclude the possibility that some metabolic changes occurred between days 1 and 6. Furthermore, we did not collect blood samples on day 7 or 14, which prevented detecting changes in l-Trp metabolites in blood. However, we previously reported that the blood level of l-Trp metabolites was unaffected by the administration of l-Trp for 21 d (1).

Conclusion

The urinary excretion amounts of l-Trp and some of its metabolites, notably KA, 3-HK, XA, 3-HA, QA, MNA, 2-Py, and 4-Py, were increased at day 7. The excretion rates of these compounds remained constant at days 14 and 21. The enzymatic metabolism of l-Trp might not have been further upregulated between days 7 and 21. By contrast, the amount of urinary excretion of 5-HT, 5-HIAA, 2-OAA, and Nam did not increase over time, even at the highest dose of l-Trp (5.0 g/d). In addition, the amount of urinary excretion of kynurenine and AnA was low. These findings indicate that administration of l-Trp for 21 d does not substantially affect metabolism of l-Trp relative to a shorter period of administration, and that l-Trp does not accumulate in healthy women.
Authors’ disclosure
The authors have no conflicts of interest to declare.

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Authors’ contributions
KS designed the study. CH and KS drafted the manuscript. CH performed the experiments. MS and TF helped design the study and the experiments. All authors read and approved the final manuscript.

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