Short Paper

Acute Toxic Effect of L-Cysteine Injected into the Peritoneal Cavity of the Rainbow Trout

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Cysteine is one of the physiologically important amino acids. However, there have been some papers describing the toxicity of cysteine to animals.^{1,2)} In our previous experiment on cysteine metabolism in fish, it was observed that cysteine gives lethal toxicity to rainbow trout when an excess amount of it is injected into the peritoneal cavity.³⁾ In this study, the effects of L-cysteine and its analogues were administrated by injection into rainbow trout and the bleeding in the gill were examined to elucidate the mechanism on the acute toxicity of L-cysteine.

Eyed eggs of rainbow trout were obtained from Fuji Trout Hatchery, Shizuoka Prefectural Fisheries Experiment Station. They were hatched out and fed with commercial diet food for trout. Fish having an average body weight of about 30 g were used for the experiments. All the experiments were conducted in water with a temperature at 15°C. The experimental fish were anesthetized with MS-222 (ethyl 3-amino benzoate, methanesulfonic acid) which was dissolved in water at a concentration of 0.01% prior to injection. A given amount of L-cysteine or its related sulfur amino acids solution was injected into the peritoneal cavity of the fish. In the cases of strong acidic substances such as L-cysteine hydrochloride, cysteinesulfonic acid and cysteic acid, the pH of the solution was adjusted to 7.0 with 5N NaOH. One milliliter of the solution per 100 g of fish body weight was injected. First of all, five levels of L-cysteine dose (2.5 to 10.0 mmol/kg body weight) were injected to each group of ten individuals to estimate LD₅₀ by the probit method.⁴⁾ The results are shown in Table 1. A large dose of cysteine led to mortality of rainbow trout with serious hemorrhage. LD₅₀ values within 2, 3, and 4 h were 7.5, 4.8, and 4.5 mmol per kg body weight, respectively.

A histological observation was made according to the standard procedure for an optical microscope.⁵⁾ Immediately after death, a variety of fish tissues were fixed in Bouin's fixative and embedded in paraffin wax. Serial sections (5 µm thick) were prepared. The sections were stained with hematoxylin-eosin. No significant histological change was observed in the tissues examined, except for the gill tissues. The most typical change in the gill tissue is shown in Fig. 1. The epithelia of secondary lamellae of the gills swelled markedly. The bloodstain was also observed at different parts of the gills. The control fish showed no

Table 1. Acute toxicity for rainbow trout of L-cysteine administered by injection into peritoneal cavity

Time (h)	LD ₅₀ (mmol/kg body weight)	95% confidence interval
2	7.5	6.3–9.6
3	4.8	4.0–5.7
4	4.5	3.7–5.3

sign of histological change. This suggests that the declined osmo-regulation function of the epithelial cells brings about the swelling of the cell, resulting in the destruction of the capillary vessel. The heavy hemorrhage might be explained by this mechanism.

Next, the acute toxicities of several cysteine analogues were examined. For the sake of convenience, the toxicity was determined by the extent of bleeding because this method is more simple and reproducible than the method using mortality. Each fish injected was kept in one l of water with aeration. Thirty min after the injection, the degree of bleeding was measured as the optical density of the water at 413 nm where hemoglobin shows its absorption maxima. The readings were corrected for the body weight of 30 g. Results are shown in Table 2. The degree of bleeding (OD at 413 nm) was almost proportional to the L-cysteine dose in the range of 3.5 to 10.0 mmol/kg of body weight. Bleeding was observed at the gills within 30 min after injection even though the dose was only 3.5 mmol/kg. This dosage is less than LD₅₀ for 4 h lethal time. D-Cysteine

Fig. 1. Histopathological change in the gill tissue of rainbow trout which was administrated with L-cysteine by injection.

A, Fish injected with 10.0 mmol L-cysteine per kg body weight; B, Control fish injected with only 0.9% saline. Bars indicate 100 µm.

Table 2. Effect of the injection of sulfur amino acids on the degree of bleeding in the gills

Amino acid	Dose* ¹	OD ₄₁₃ * ²
L-Cysteine	3.5	0.01 ± 0.00
	5.0	0.08 ± 0.02
	7.1	0.07 ± 0.03
	10.0	0.22 ± 0.05
D-Cysteine	5.0	0.06 ± 0.02
	10.0	0.19 ± 0.06
N-Acetyl-L-cysteine	10.0	0.00 ± 0.00
S-Methyl-L-cysteine	10.0	0.03 ± 0.01
L-Cysteinesulfinic acid	10.0	0.00 ± 0.00
L-Cysteic acid	10.0	0.00 ± 0.00
Hypotaurine	10.0	0.00 ± 0.00
Taurine	10.0	0.00 ± 0.00
Glutathione	4.0	0.02 ± 0.00
L-Methionine	4.0	0.00 ± 0.00

*¹ mmol dose per kg body weight.*² The degree was expressed as the optical density of rearing water at 413 nm. Values are means of eight individual measurement ± standard error.

showed similar toxic effect to that of L-cysteine. *N*-Acetyl-L-cysteine showed hemorrhagic effect but *S*-methyl-L-cysteine did not. Other sulfur amino acids cysteinesulfinic acid, hypotaurine, and taurine involved in the cysteine catabolism also showed no hemorrhagic effect (Table 2). Large doses of methionine and glutathione which is a tripeptide having free SH group could not be administrated to fish due to their poor solubilities in water. However, glutathione showed a slight toxic effect even in a small dose. Neither methionine nor cysteic acid showed any effect. These findings indicate that the sulfhydryl group in

the molecule might be involved in toxicity to rainbow trout.

Cysteine dioxygenase catalyzes the oxygenation of cysteine to form cysteinesulfinic acid which is the first metabolite in the cysteine catabolism to taurine or sulfate.⁶⁾ The activity of this enzyme in the rainbow trout liver was induced within several hours after the administration of L-cysteine.³⁾ Moreover, L-cysteinesulfinic acid showed no toxic effect for rainbow trout in this study. These facts suggest that cysteine dioxygenase plays a role of the detoxication of cysteine in rainbow trout, *i.e.* L-cysteine may be metabolized into non-toxic L-cysteinesulfinate by cysteine dioxygenase. Cysteine concentration in liver was found to be kept at a low level in rainbow trout even if a large amount of cystine was taken in through feed.⁷⁾ This fact also reflects the rapid turnover of cysteine in the rainbow trout liver.

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