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Increase in tissue cysteine level and excretion of sulfate and taurine after intragastric administration of L-2-oxothiazolidine-4-carboxylate in rats

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Summary. Five mmol of L-2-oxothiazolidine-4-carboxylate (OTC)/kg of body weight was administered into the stomach of rats, and cysteine levels in tissues and sulfate and taurine excreted in the urine were determined. The cysteine (plus cystine expressed as cysteine) concentration in the liver increased to 170–200% of the original level at 30 min and that in the blood to 160% at 60 min after the OTC administration. These high levels were maintained until 8 h after the administration and decreased gradually thereafter. Excretion of sulfate and taurine increased after the OTC administration and the increase corresponded to 26% and 15%, respectively, of the OTC administered. These findings suggest that at least about 40% of the OTC administered into the stomach was taken up and converted to cysteine, which was metabolized to sulfate and taurine.

Keywords: Amino acids – L-2-Oxothiazolidine-4-carboxylate – Cysteine precursor – Cysteine metabolism – Sulfate – Taurine

Introduction

L-2-Oxothiazolidine-4-carboxylate (OTC) is a derivative of L-cysteine and is converted to L-cysteine by 5-oxoprolinase reaction (Williamson and Meister, 1981 and 1982). The administration of OTC resulted in the increase in hepatic glutathione levels in mice (Williamson and Meister, 1981; Williamson et al., 1982; Meister et al., 1986), chick and rats (Thau et al., 1989), and guinea pigs (Nishina et al., 1987; Ubuka et al., 1990). It has been shown in humans that oral administration of OTC resulted in the appearance of OTC in blood plasma and in the increase in the concentration of cysteine in blood plasma and of cysteine and glutathione in lymphocytes (Porta et al., 1991). These findings suggest that OTC is metabolized to cysteine and utilized to glutathione synthesis in these animals and humans.

L-Cysteine is mainly metabolized to inorganic sulfate and taurine in mammals and excreted in the urine (Roy and Trudinger, 1970; Griffith, 1987). Our previous studies have shown that the increase in the excretion of inorganic sulfate and taurine after the intragastric administration of L-cysteine to rats corresponded to 95% of the L-cysteine administered (Yoshida et al., 1989) and that the intraperitoneal administration of OTC to rats resulted in a 3-fold increase in liver taurine concentration (Taguchi et al., 1990). The present study was undertaken to examine the effect of oral administration of OTC on the tissue cysteine levels and the excretion of sulfate and taurine in rats in order to confirm how effectively the orally administered OTC is utilized as the cysteine precursor.

Materials and methods

Materials

Male Wistar rats were used and maintained on a laboratory diet MF of Oriental Yeast Company, Tokyo, Japan. The body weights were 250 to 300g at the time of OTC administration. OTC was obtained from Chemical Dynamics Corp., South Plainfield, NJ, U.S.A.

Feeding rats with a synthetic diet and administration of OTC

One week before the OTC administration, the diet was changed to a synthetic 25% casein diet (Yoshida et al., 1989), in which sulfate was omitted from the original formula. Each rat was fed this diet and water ad libitum in a separate cage. OTC solution (0.5 mmol/ml) was neutralized with sodium hydroxide solution and administered into the stomach of rats using a catheter at a dose of 5 mmol/kg of body weight. This method of intragastric administration was used in this study in order to administer a known amount of OTC at a specific time.

Determination of cysteine in tissues

At the appropriate time after the OTC administration, rats were killed by decapitation after ether anesthesia. Blood was collected in a beaker containing 200 units of heparin and chilled in an ice bath. The blood was homogenized in 3 volumes of 6.7% perchloric acid. Liver and kidney were washed with cold (0°C) 0.9% sodium chloride solution and homogenized as above. Homogenates were centrifuged at 10,000 × g for 30 min. Cysteine and cystine concentrations in the supernatants were determined by acidic ninhydrin reaction (Gaitonde, 1967).

Determination of sulfate and taurine in the urine

Twenty-four-hour urine was collected in an Erlenmeyer flask containing 5 ml of 50% acetic acid and 1 ml of toluene. Free sulfate was determined using 1:50 diluted urine with an ion chromatograph (Ubuka et al., 1992). Total sulfate (free plus ester sulfate) was determined after hydrolysis of the diluted urine in 0.4 M hydrochloric acid at 80°C for 2 h. Taurine was determined by ninhydrin reaction after separation using ion-exchange columns as reported (Yoshida et al., 1989).

Statistical analysis

The statistical significance was assessed by Student's t test or paired t test as shown below.

Results

Cysteine concentration in blood, liver and kidney after the intragastric administration of OTC

In the blood of rats used in the present study, 33 ± 9 nmol/ml of cysteine, 52 ± 4 nmol/ml of half cystine (cystine expressed as cysteine), and thus 85 ± 5 nmol/ml of total cysteine (cysteine + half cystine) were present. The total cysteine contents increased rapidly after the intragastric administration of OTC and the values were 132 ± 9 and 136 ± 10 nmol/ml of blood at one and two h after the administration. As shown in Fig. 1, this high value was maintained for at least 7 h. The cysteine contents decreased thereafter and returned to the original level at about 12 h after the OTC administration.

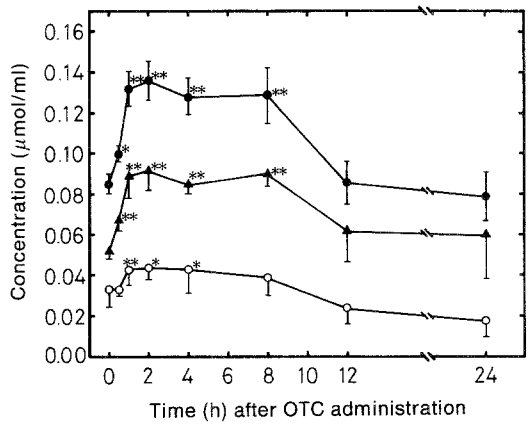

Fig. 1. Changes in the cysteine contents in the blood of rats after the intragastric administration of 5 mmol of L-2-oxothiazolidine-4-carboxylate (OTC) per kg of body weight. Each value is the mean ± SD obtained from 5 rats. Cysteine, o—o; half cystine (cystine expressed as cysteine), ▲—▲; total cysteine, sum of cysteine and half cystine, ●—●. Significant difference from the value at 0 h was assessed by Student's *t* test and is shown with asterisks: **, $p < 0.01$; *, $p < 0.05$

The content of cysteine in the liver was 136 ± 40 nmol/g of fresh tissue and that of the total cysteine content was 138 ± 29 nmol/g. The cysteine content increased rapidly after the OTC administration as shown in Fig. 2. At 30 min after the administration, the total cysteine content was 258 ± 17 nmol/g and this high value was maintained until 4 h after the OTC administration. Then, the cysteine content decreased gradually and reached the original level at 24 h after the administration.

The cysteine content in the kidney was 774 ± 230 nmol/g and that of half cystine was 214 ± 114 nmol/g of fresh tissue. As shown in Fig. 3, the cysteine content did not increase as remarkably as that seen in the blood and liver. Only significant increase was the total cysteine content of 1,163 ± 125 nmol/g at 2 h after the OTC administration.

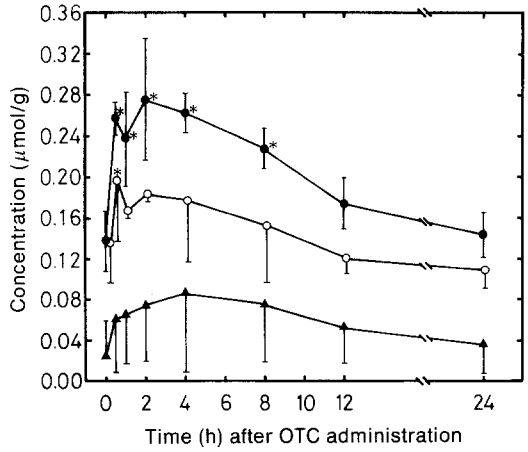

Fig. 2. Changes in the cysteine contents in the liver of rats after the intragastric administration of 5 mmol of L-2-oxothiazolidine-4-carboxylate (OTC) per kg of body weight. Each value is the mean ± SD obtained from 5 rats. Symbols are the same as those in Fig. 1

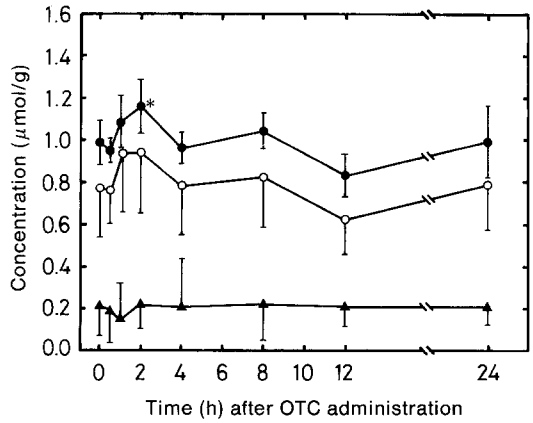

Fig. 3. Changes in the cysteine contents in the kidney of rats after the intragastric administration of 5 mmol of L-2-oxothiazolidine-4-carboxylate (OTC) per kg of body weight. Each value is the mean ± SD obtained from 5 rats. Symbols are the same as those in Fig. 1

Sulfate excretion after the intragastric administration of OTC

As shown in Fig. 4, rats in the present experiments excreted approximately 1 mmol/kg of body weight of total (free plus ester) sulfate per day. The total sulfate excreted during 24 h on the 7th day, the previous day of OTC administration, was 1.212 ± 0.221 mmol/kg. Free sulfate excreted on the same day was 1.101 ± 0.226 mmol/kg per day, showing that 91% of the urinary sulfate excreted was in the free form.

Total and free sulfate excreted on the 8th day during 24 h after the administration of 5 mmol of OTC /kg of body weight were 2.558 ± 0.407 and 2.413 ± 0.459 mmol/kg per day, respectively. Thus, free sulfate constituted 94% of the total excretion of sulfate. The increment of the excretion of total sulfate to that on the previous day was 1.346 mmol/kg/day, corresponding to 27% of the

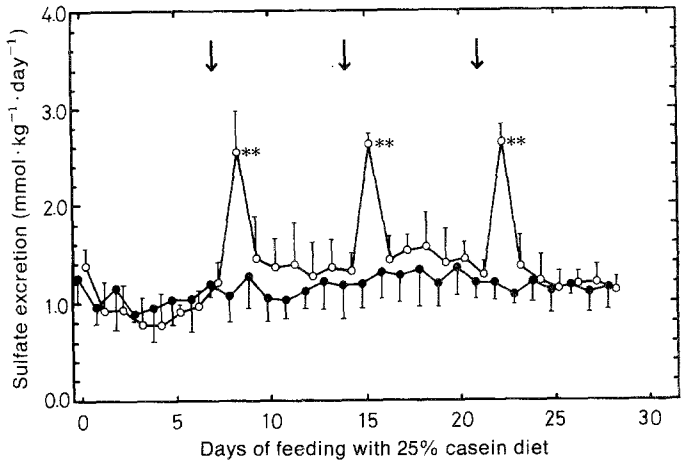

Fig. 4. Excretion of sulfate in the urine of rats after the intragastric administration of L-2-oxothiazolidine-4-carboxylate (OTC). Total (free plus ester) sulfate in 24-h urine beginning from 9:00 a.m. was determined by ion chromatography. Five mmol of OTC per kg of body weight was administered at 9:00 a.m. which was indicated with arrows. Each value is mean ± SD obtained from 5 animals. OTC-administered rats, o—o; control rats, ●—●. Significant difference from the value on the previous day of OTC administration was assessed by paired *t* test and is shown with asterisks: **, $p < 0.01$

sulfur of the OTC administered. Excretion of total and free sulfate in the following 24 h was 1.457 ± 0.329 and 1.351 ± 0.326 mmol/kg per day, respectively. The increment of the total sulfate excreted in this period corresponded to 2.7% of the sulfur administered.

The same type of OTC administration as described above was repeated two more times as shown in Fig. 4. Results were almost identical. On the average, the increase in the sulfur excretion in the form of free and ester sulfate during 24 h after the OTC administration corresponded to 26% of the sulfur administered in the form of OTC.

Taurine excretion after intragastric administration of OTC

Taurine excretion in the urine was examined in the same rats in which sulfate excretion was studied. Average taurine excretion in rats which were fed the commercial MF diet was 376.3 ± 137.1 μmol/kg of body weight/day. The taurine excretion decreased by conversion of the diet to the 25% casein diet, and the excretion on the 7th day after the diet change, the previous day of the OTC administration, was 121.3 ± 56.0 μmol/kg/day.

As shown in Fig. 5, the excretion of taurine increased to 364.4 ± 97.9 μmol/kg/day after the administration of 5 mmol of OTC/kg of body weight. In contrast to the sulfate excretion, the increased taurine excretion continued for 4 days after the OTC administration and then returned to the basal level. On the 2nd and 3rd OTC administration, the taurine excretion increased likewise and the increased taurine excretion continued for 3 or 4 days. The average increment of the total taurine excretion during these periods after the OTC administration was 758.5 μmol, corresponding to 15% of the OTC administered. Thus, the

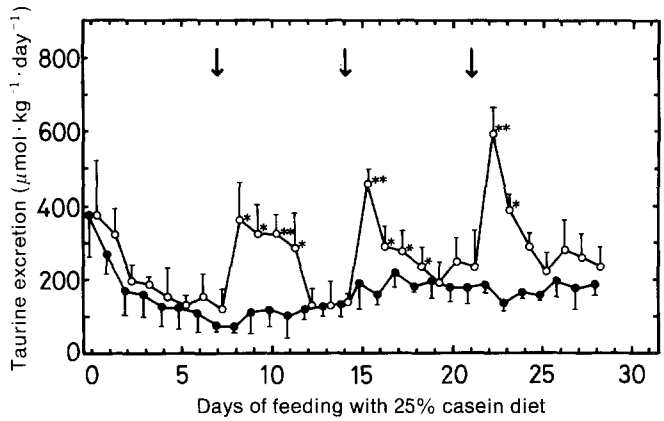

Fig. 5. Excretion of taurine in the same urine of the rats as that in Fig. 4. Taurine in the 24-h urine of rats received L-2-oxothiazolidine-4-carboxylate (o—o) and that of the control rats (●—●) was determined. Each value is mean ± SD obtained from 5 animals. Significant difference from the value on the previous day of OTC administration was assessed by paired *t* test and is shown with asterisks: **, $p < 0.01$; *, $p < 0.05$

average increase in the excretion of the sulfur in the form of total sulfate and taurine after the intragastric administration of OTC to rats corresponded to 41% of the sulfur of OTC administered under the present conditions.

Growth curves of rats received intragastric administration of OTC

Fig. 6 shows the growth curves of rats which received OTC administration and that of control rats. Both curves are essentially the same. The average increase in the body weights of rats received OTC were slightly higher than that of the

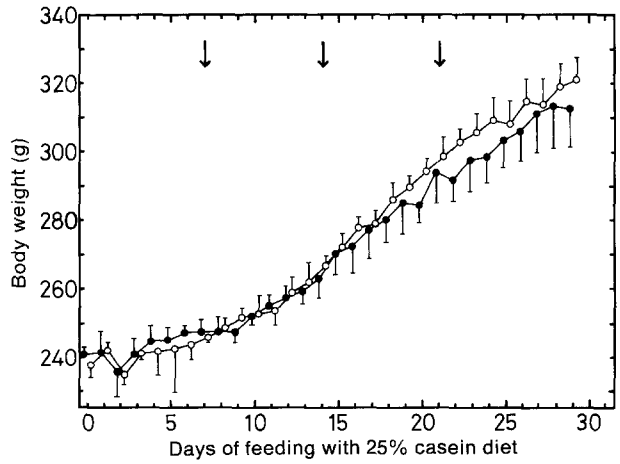

Fig. 6. Growth curves of the same rats as those of Figs. 4 and 5. Rats received L-2-oxothiazolidine-4-carboxylate, o—o; control rats, ●—●. Arrows indicate OTC administration. There was no significant difference between two groups, which was assessed by Student's *t* test

control rats after the 3rd administration of OTC, but the difference was statistically insignificant.

Discussion

It has been shown that OTC is a good substrate for 5-oxo-L-prolinase (EC 3.5.2.9) and is converted to cysteine (Williamson and Meister, 1981, 1982). It has been reported that the intraperitoneal injection of OTC resulted in the increase in hepatic glutathione in mice (Williamson and Meister, 1981; Williamson et al., 1982; Meister et al., 1986). Oral ingestion of OTC also resulted in the increase in the hepatic glutathione concentration in chicks and rats (Thau et al., 1989) and in human lymphocytes (Porta et al., 1991). Our previous experiments have shown that the glutathione concentration in the guinea pig liver also increased after the intraperitoneal injection of OTC (Nishina et al., 1987; Ubuka et al., 1990) and that the intraperitoneal injection of OTC to rats resulted in the increase in the hepatic taurine contents and in the urinary taurine excretion (Taguchi et al., 1990). All these findings indicate that OTC is converted to cysteine when administered intraperitoneally or orally to these animals and humans.

In the present study, it was shown that the intragastric administration of OTC resulted in the increase in the cysteine and cystine contents in the liver and blood. Fig. 7 compares the fluctuation of the total cysteine contents in the liver, blood and kidney after the administration of OTC. The rapid increase occurred in the liver, followed by the blood. The increase in these tissues was statistically significant over the range from 30 min to 8 h after the OTC administration. The increased level of the total cysteine contents in the liver decreased more rapidly than that in the blood. It is unknown where the orally administered OTC is

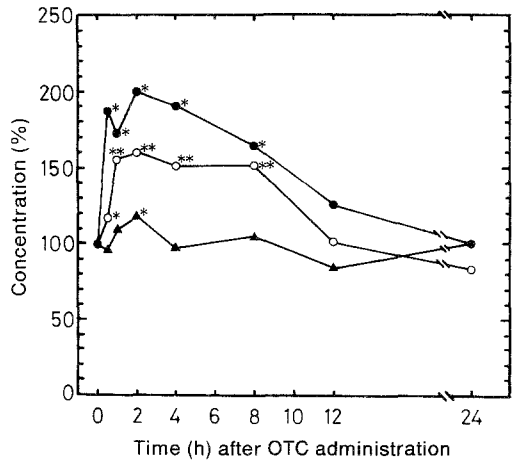

Fig. 7. Comparison of the changes in the total cysteine contents in the blood (o—o), liver (●—●) and kidney (▲—▲) of rats after the intragastric administration of 5 mmol of L-2-oxothiazolidine-4-carboxylate (OTC) per kg of body weight. Each value is the mean obtained from 5 animals. Significant difference is shown with asterisks as that in Fig. 1

converted to cysteine. The conversion might occur in the intestinal rumen or in enterocytes (Thau et al., 1989). However, the present findings, together with that of Porta et al. (Porta et al., 1991), seem to suggest that at least some OTC was absorbed from the intestine and transported through the portal vein to the liver, in which a substantial 5-oxoprolinase activity was present (Van Der Werf et al., 1975), and that cysteine formed from OTC in the liver was transported to the blood.

Previously, we reported that the intragastric administration of L-cysteine to rats resulted in the increase in the excretion of sulfate and taurine (Yoshida et al., 1989). The increased excretion of free sulfate and taurine in the 24-h urine after the cysteine administration corresponded to 80% and 15%, respectively, of the cysteine administered, showing that rats excreted sulfur corresponding to 95% of the L-cysteine administered and that a sulfur balance existed between the sulfur taken and excreted. We also reported that the intraperitoneal administration of OTC resulted in the increase in taurine excretion, which corresponded to approximately 10% of the OTC administered (Taguchi et al., 1990).

In the present study, it was shown that the increase in the sulfate excretion during 24 h after the intragastric administration of OTC corresponded to 26% of the sulfur administered as OTC, and that in the taurine excretion during 3 to 4 days after the OTC administration corresponded to 15% of the sulfur administered. These results indicate that at least about 40% of the OTC administered into the stomach was utilized in rats and metabolized to sulfate and taurine. As the direct administration of OTC into the stomach is considered to be equivalent to its oral administration, the present findings indicate that the orally administered OTC is a good precursor of cysteine and that the efficiency of the orally administered OTC as the cysteine precursor was approximately 40% of the cysteine administered. Thau et al. reported a similar conclusion, in which the orally ingested OTC in chicks and rats was active as a cysteine precursor as judged by the effect for growth and hepatic glutathione biosynthesis, and OTC was slightly inferior to cysteine in rats (Thau et al., 1989). As mentioned above, it was shown that the oral administration of OTC in humans resulted in the increase in the plasma cysteine level (Porta et al., 1991). Thus, it may be concluded that OTC taken orally is an effective precursor for L-cysteine and thus the oral administration is an effective route of OTC administration in animals and humans.

References

Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of naturally occurring amino acids. Biochem J 104: 627–633

Griffith OW (1987) Mammalian sulfur amino acid metabolism: An overview. In: Jakoby WB, Griffith OW (eds) Methods in enzymology vol 143. Academic Press, New York, pp 366–376

Meister A, Anderson ME, Hwang O (1986) Intracellular cysteine and glutathione delivery systems. J Am Coll Nutr 5: 137–151

Nishina H, Ohta J, Ubuka T (1987) Effect of L-2-oxothiazolidine-4-carboxylate administration on glutathione and cysteine concentrations in guinea pig liver and kidney. Physiol Chem Phys Med NMR 19: 9–13

Porta P, Aebi S, Summer K, Lauterburg H (1991) L-2-Oxothiazolidine-4-carboxylic acid, a cysteine prodrug: pharmacokinetics and effects on thiols in plasma and lymphocytes in humans. J Pharmacol Exp Ther 257: 331–334

Roy AB, Trudinger PA (1970) The biochemistry of inorganic compounds of sulphur. Cambridge University Press, Cambridge, pp 289–316

Taguchi T, Akagi R, Ubuka T (1990) Tissue contents and urinary excretion of taurine after administration of L-cysteine and L-2-oxothiazolidine-4-carboxylate to rats. Acta Med Okayama 44: 123–128

Thau KC, Funk MA, Baker DH (1989) L-2-Oxothiazolidine-4-carboxylate as a cysteine precursor: efficacy for growth and hepatic glutathione synthesis in chicks and rats. J Nutr 120: 158–165

Ubuka T, Ohta J, Akagi R, Nishina H, Yoshida S, Taguchi T (1990) Changes in glutathione and cysteine levels after 2-oxothiazolidine-4-carboxylate administration in guinea pig tissues. In: Lubec G, Rosenthal GA (eds) Amino acids. Chemistry, biology and medicine. Escom, Leiden, pp 951–955

Ubuka T, Ohta J, Yao W-B, Abe T, Teraoka T, Kurozumi Y (1992) L-Cysteine metabolism via 3-mercaptopyruvate pathway and sulfate formation in rat liver mitochondria. Amino Acids 2: 143–155

Van Der Werf P, Griffith OW, Meister A (1975) 5-Oxo-L-prolinase (L-pyroglutamate hydrolase) Purification and catalytic properties. J Biol Chem 250: 6686–6692

Williamson JM, Boettcher B, Meister A (1982) Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. Proc Natl Acad Sci USA 79: 6246–6249

Williamson JM, Meister A (1981) Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-prolinase substrate. Proc Natl Acad Sci USA 78: 936–939

Williamson JM, Meister A (1982) New substrate of 5-oxoprolinase. J Biol Chem 257: 12039–12042

Yoshida S, Akagi R, Ubuka T (1989) Sulfate and taurine excretion in rats after L-cysteine administration. Acta Med Okayama 43: 281–288

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