Effects of L-2-oxothiazolidine-4-carboxylate, a Cysteine Pro-drug, on Teratogenicity of 5-fluorouracil in Mice

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ABSTRACT

Embryotoxicity and teratogenicity of 5-fluorouracil (5-FU) and modulation of its effect by L-2-oxothiazolidine-4-carboxylate (OTC), a cysteine pro-drug, were evaluated in mice. Pregnant ICR mice were intraperitoneally (i.p.) injected with 25 mg/kg of 5-FU on day 11 of gestation (vaginal plug = day 0). Mice were pretreated i.p. with 950 mg/kg of OTC 4 hours before dosing with 5-FU. Dams were killed on day 17 of gestation. Fetuses were examined for external malformations, especially limb malformations. Pretreatment with OTC decreased the frequency and severity of oligodactyly induced by 5-FU, although the differences were not significant statistically. There was little difference in either liver glutathione levels, or body weight gain during gestation of dams between the 5-FU group and the 5-FU plus OTC group. Fetal mortality and fetal weight of the group treated with 5-FU alone were comparable with those of the group pretreated with OTC. In the present study, teratogenicity of 5-FU seemed to be slightly mitigated with OTC pretreatment.

Key words: *Teratogenicity, Modification, 5-fluorouracil, Glutathione*

Glutathione (GSH), γ-glutamylcysteinylglycine, is the most abundant nonprotein thiol in cells. It is known to be a major intracellular antioxidant, a key component in the metabolism of cysteine and cysteine-containing proteins, and a specific deactivator of potentially toxic electrophilic agents. L-2-oxothiazolidine-4-carboxylate (OTC), a cysteine pro-drug, stimulates formation of GSH in the liver. Therefore, the intracellular GSH levels in different cell systems can be increased after treatment with OTC[13].

Teratogenicity of the antitumor agent 5-fluorouracil (5-FU) has been characterized by inducing limb malformations in mice[2,3,6]. There is a considerable advantage in using limb malformations to evaluate the protective or augmentative effect of chemicals on teratogenicity because limb malformations can be easily defined by the number of missing digits. We have previously shown that GSH depletion with diethylmalate increases the incidence of oligodactyly. On the contrary, pretreatment with GSH decreases the incidence of oligodactyly induced with 5-FU in mice[8]. We have also reported that GSH depletion with phorone and/or buthionine sulfoximine increased the frequency and severity of oligodactyly induced with 5-FU in mice[9].

This is a preliminary report of the protective effect of OTC on 5-FU teratogenicity in mice.

METERIALS AND METHODS

Virgin ICR mice, 10 weeks old, were purchased from Shizuoka Laboratory Animal Center. They were kept under conditions of regular light-dark cycles (light between 7 A.M. and 7 P.M.), at a temperature of 24 ± 2°C, and were given standard lab chow (MBR-1, Funabashi Farm) and drinking water ad libitum for the duration of the experiment. After acclimatization for 1 week, the animals were allowed to mate overnight. Females were examined for the presence of a copulatory plug the following morning, which was designated as day 0 of gestation.

The chemicals for injection were dissolved in the following solutions in a volume of 5 ml/kg body weight; GSH and 5-FU (Kyowa Hakko Co., Ltd.) in physiological saline, OTC (Aldrich Chemical Co.,) in physiological saline and titrated to pH 7.0 to 8.0 with NaOH.

As a preliminary experiment, levels of GSH in pregnant mice were determined after intraperitoneal (i.p.) administration of OTC 950 mg/kg body weight on day 11 of gestation. Pregnant mice were anesthetized with ether and 1 ml of blood was taken from the femoral artery. The liver was removed at 0, 2, 4, 6 or 24 hours after administration of OTC. Animals were killed at the time of blood sampling. Serum and supernatant fluid of

homogenized liver were measured for GSH levels by use of Hitachi model 655 high performance liquid chromatography modifying Hissin and Hilf's original method[5] of fluorescing reaction of GSH with o-phthalaldehyde. Levels of cystine in the liver treated with OTC 950 mg/kg were also determined. Cystine levels were measured instead of cysteine levels, because cysteine levels could not be measured in our laboratories. Cystine levels were determined with the ninhydrin reagent by the method of high resolution protein hydrolyzate analysis[11] (Hitachi model 835 amino acid analyzer).

The teratogen, 5-FU, was i.p. injected at a single dose level of 25 mg/kg body weight on day 11 of gestation. Control animals were injected i.p. with 5 ml/kg of physiological saline. A cysteine pro-drug, OTC, 950 mg/kg i.p. was administered 4 hours before the 5-FU treatment, in accordance with the report of Williamson and Meister[13].

Pregnant mice were killed on day 17 of gestation, when gross examination of limbs could be done efficiently. The uterine horns were exposed and the number of implants, resorptions, and live fetuses were counted. Live fetuses were removed
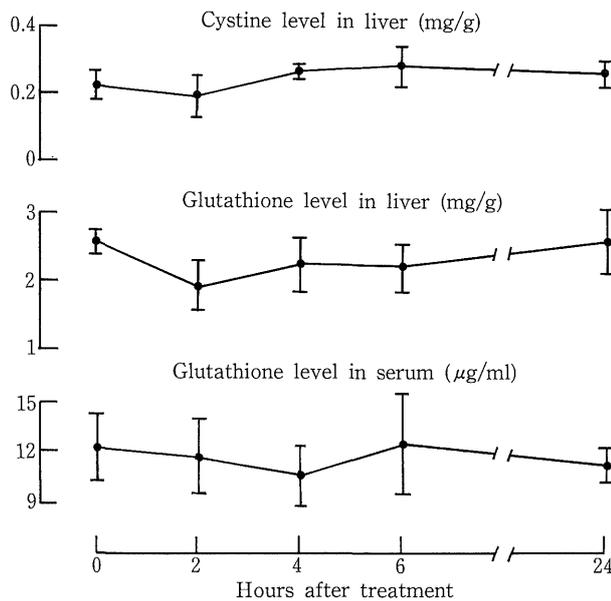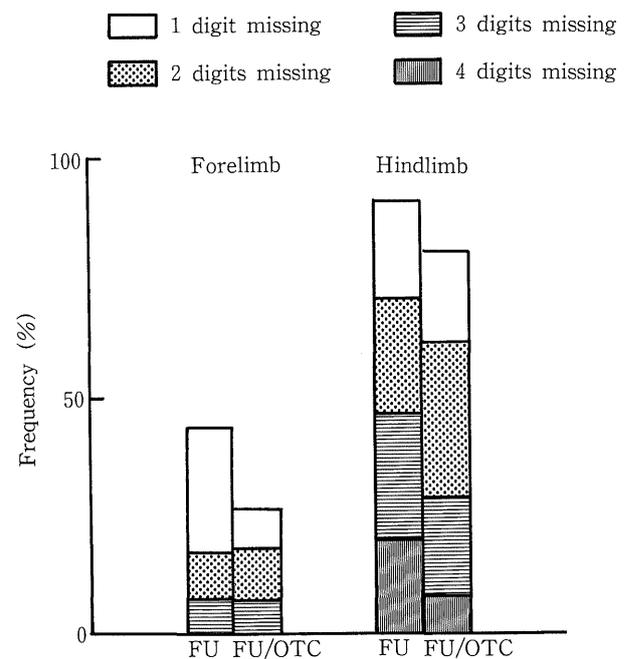

Fig. 1. Effects of 1-2-oxothiazolidine-4-carboxylate (OTC) on glutathione levels in maternal liver and serum, and cystine levels in maternal liver of ICR mice. OTC 950 mg/kg i.p. was administered on day 11 of gestation. Pregnant mice were killed at the times indicated. Levels of glutathione were determined by high performance liquid chromatography, and levels of cystine by an amino acid analyzer. Data are shown as the mean ± S.D. of 4—5 mice.

Fig. 2. Effects of 1-2-oxothiazolidine-4-carboxylate (OTC) on severity of limb anomalies induced with 5-fluorouracil (5-FU) in mice. Pregnant ICR mice were injected i.p. with 25 mg/kg of 5-FU on day 11 of gestation. OTC 950 mg/kg i.p. was injected 4 hours before the 5-FU treatment. Severity of oligodactyly was evaluated by the number of missing digits.

Table 1. Effects of OTC on the teratogenicity of 5-fluorouracil in mice[1]

	Saline i.p.	OTC 950 mg/kg i.p.	5-FU 25 mg/kg i.p.	OTC 950 mg/kg i.p. and 5-FU 25 mg/kg i.p.
No. of dams	11	12	10	12
Initial maternal body weight (day 11, g)	50.1 ±4.0	47.3 ±3.7	48.4 ± 4.7	49.8 ± 3.3
Final maternal body weight (day 17, g)	69.0 ±6.0	66.7 ±4.2	61.4 ± 4.9	63.0 ± 4.0
Maternal body weight gain (g)	18.9 ±4.6	19.4 ±2.8	13.0 ± 3.2	13.2 ± 2.3
No. of implantations per litter	15.6 ±3.7	15.7 ±1.5	13.9 ± 3.3	16.2 ± 2.6
Average fetal mortality (%)	12.4 ±8.6	13.0 ±9.3	12.5 ±14.8	9.0 ± 8.6
No. of live fetuses per litter	13.9 ±3.8	13.6 ±1.6	12.2 ± 3.4	14.6 ± 1.8
Average fetal weight (g)	1.05±0.10	1.09±0.07	0.75± 0.09**	0.80± 0.11**
% limb anomalies				
Forelimb	0	0	43.4 ±31.6	26.4 ±28.8
Hindlimb	0	0	90.6 ±13.5	80.2 ±20.2
Average No. of missing digits				
Forelimb	0	0	0.7 ± 0.5	0.5 ± 0.6
Hindlimb	0	0	2.2 ± 0.9	1.8 ± 0.7

[1] Values shown are means ± standard deviations, excluding those for No. of dams.
** $p < 0.01$ vs. Saline

from the uterus, weighed, and examined for external malformations.

Analysis of variance (ANOVA) and Dunnett's test for multiple comparisons were employed for the analysis of: GSH levels in serum and liver of the dams, cystine levels in the liver, the weight gain of dams during gestation, the number of implants, the number of live fetuses, and the weight of live fetuses. For the fetal mortality and incidence of limb malformations, analysis of variance (ANOVA) and Kruskal-Wallis's test were used. For analysis of severity of limb anomalies, the number of missing digits was used as the indicator, and the above statistical tests were applied. The litter was designated as the statistical unit by computing the mean value of data in each litter.

RESULTS

Figure 1 shows the results of this preliminary study to determine the cystine levels in the maternal liver, or the GSH levels in the maternal liver, as well as the serum after administration of OTC. Cystine levels in the liver had a tendency to increase after treatment with OTC alone; however, the differences were not significant. The GSH levels in the liver or serum did not change after treatment with OTC.

The influence of OTC on the teratogenicity of 5-FU is shown in Table 1, and the severity of limb malformations is shown in Figure 2. A cysteine pro-drug, OTC itself produced no adverse effects on the dams or fetuses. There was little difference in body weight gain of dams during gestation period among the groups treated with 5-FU alone and 5-FU with OTC. In the group treated with OTC and 5-FU, fetal mortality and fetal weight were comparable with the group treated with 5-FU alone.

The limb abnormalities induced with 5-FU under the present condition were oligodactyly only. Pretreatment with OTC decreased the incidence and the severity of 5-FU induced oligodactyly in both forelimb and hindlimb. The differences were not, however, significant statistically.

DISCUSSION

It is known that GSH participates in a number of fundamental biological processes, including the syntheses of DNA and proteins, the transport of some amino acids, enzyme activity, metabolism, and cell defense against a variety of internal and external stress[7]. It has also been reported that GSH has a protective effect against certain teratogens. Ashby et al[1] observed that exogenous GSH decreased the incidence of malformations in rats induced by cyclophosphamide or chlorambucil. The inhibitory effect of GSH or cysteine against the teratogenicity and mutagenicity of cyclophosphamide was reported by Hales[4].

Previously[8], we demonstrated that exogenous GSH decreased the incidence of limb malformations induced with 5-FU in mice and rats. In the present study, OTC, a cysteine pro-drug, seemed to slightly mitigate the 5-FU induced teratogenicity. The GSH levels in the liver or serum were not changed. However, the cystine levels in the liver had a tendency to increase after treatment with OTC. The 5-FU induced teratogenicity might be mitigated by cysteine, a reduced type of cystine, or OTC itself, because of a tendency to increase the cystine levels in the liver treated with OTC. The acetoaminophen induced lethal effects in mice were protected by OTC[14] and doxorubicin induced cytotoxicity in chinese hamster V79 cells were also protected by OTC[10]. Pretreatment with N-acetylcysteine, a precursor of GSH, reduced the incidence of diphenylhydantoin-induced cleft palate in mice[12].

Feeding condition might conceivably influence the result. Williamson and Meister[13] used mice which were fasted overnight, while our mice were fed. It may have been more preventive if we had used fasted mice, or more effective precursors of GSH, such as N-acetylcysteine. We are planning to study the modification of the 5-FU induced teratogenicity by N-acetylcysteine in mice.

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