Effect of CPT-11 on Lipid Peroxide Level in Mouse Tissues

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We examined the effects of CPT-11 on lipid peroxide level and glutathione peroxidase (GSHpx) activity as indices of the toxicity of this antitumor agent in mouse and rat tissues. After CPT-11 (100 mg/kg, i.p.) administration, the lipid peroxide level in the heart increased 1.5 fold (mice) and 1.3 fold (rats) over the control levels. GSHpx activity decreased to 64% of the control. In the lung, the lipid peroxide level and GSHpx activity increased 2.5 fold and decreased to 74% after CPT-11 administration, respectively, compared with the control values. These results suggested that CPT-11 may cause cardiotoxicity and pulmototoxicity. In rat bone marrow, the lipid peroxide level increased on the 2nd day after CPT-11 injection. We suggest that in both single and combination treatment with CPT-11, the possibility of side effects should be taken into consideration.

Key words: CPT-11 — Lipid peroxide — Antitumor agent — Glutathione peroxidase — Side effect

Irinotecan hydrochloride (CPT-11), a water-soluble derivative of camptothecin, is an antitumor alkaloid contained in *Camptotheca acuminata*.¹⁾ CPT-11 has higher antitumor activity and lower toxicity than camptothecin.²⁾ It has been commercially available for the chemotherapy of lung carcinoma since 1994, and acts by inhibiting topoisomerase I activity and suppressing DNA biosynthesis in the tumor cell.^{2–5)} Nevertheless, CPT-11 has severe toxicity, causing bone marrow suppression and diarrhea,⁶⁾ and its clinical use is strictly controlled. It has been reported that lipid peroxide levels are increased in plasma and tissues of patients with geriatric diseases^{7–10)} and the tissues after administration of various drugs.^{11, 12)} We previously suggested^{13–15)} that the increase in lipid peroxides induced by adriamycin may be related to its cardiotoxicity.^{16, 17)} However, there has been no report on changes in lipid peroxide levels in the tissues after CPT-11 treatment. Therefore, we examined the changes in lipid peroxide level and glutathione peroxidase (GSHpx) activity¹⁸⁾ after CPT-11 administration in order to clarify the mechanism of the toxicity of CPT-11.

MATERIALS AND METHODS

Reagents CPT-11, in 100 mg/5 ml vials (“Topotecan”), and adriamycin, in 10 mg vials (“Adriacin”), were purchased from Daiichi Pharmaceutical Co., Ltd., Tokyo and Kyowa Fermentation Inc., Tokyo, respectively. 2-Thiobarbituric acid was purchased from Wako Pure Chemical Industries Co., Ltd., Tokyo. β -Nicotinamide adenine dinucleotide phosphate, reduced form (NADPH, type I), adenosine 5'-diphosphate (ADP, grade I) and glutathione, reduced form, were purchased from Sigma Chemical Co., St. Louis, MO.

Animals Male CDF₁ mice (6 weeks old and weighing 20–25 g) and male Wistar rats (6 weeks old and weighing 100–120 g) were obtained from Japan SLC Inc., Hamamatsu. The animals were housed in a room maintained at $25 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ relative humidity and were given free access to standard laboratory feed and water. CPT-11 (100 mg/kg, i.p.) was injected into the mice. The animals were killed by cervical dislocation at specific times within 6 days of CPT-11 administration. The lung, heart, liver and kidney were rapidly dissected out and washed in ice-cold isotonic saline. Each tissue was blotted and weighed to obtain the wet weight. The tissue samples were homogenized. Determinations of lipid peroxide level and GSHpx activity in the samples were carried out according to our procedure¹⁹⁾ and according to Hafeman *et al.*,²⁰⁾ respectively. In another experiment, CPT-11 (60 mg/kg, i.p.) was injected into rats and determinations of the lipid peroxide level in tissues and thigh bone marrow were carried out. The protein levels in the tissue samples were determined according to the method of Lowry *et al.*²¹⁾

NADPH-dependent lipid peroxidation test NADPH-dependent lipid peroxidation in the microsomal fraction of mouse liver was assayed according to Svingen *et al.*²²⁾ In brief, the reaction mixture contained liver microsomes (0.5 mg protein/ml), 1.7 mM ADP and 0.1 mM FeCl_3 in 0.05 M Tris-HCl buffer (pH 7.5). CPT-11 was added. Lipid peroxidation in the reaction mixture was started by the addition of 0.1 mM NADPH and incubation was conducted at 37°C . The reaction mixture in the control group contained 0.05 M Tris-HCl buffer (pH 7.5) instead of CPT-11. After incubation for 60 min, the lipid peroxide level in the reaction mixture was determined.

Statistical analysis Statistical analysis was done by using ANOVA and Student's *t* test.

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RESULTS

Changes in lipid peroxide level and GSHpx activity in mouse tissues after CPT-11 administration The body weight of CPT-11-treated mice transiently decreased to 95% of the level before CPT-11 administration and then did not change during the experimental period. Lipid peroxide levels in the heart (Fig. 1) gradually increased, reaching 1.5 times ($P < 0.01$) the control level (1.78 ± 0.36 nmol/mg protein) on the 4th and 5th days after administration. GSHpx activity in the heart transiently decreased to 71.1% ($P < 0.001$) and 64.9% ($P < 0.01$) of the control activity (12.69 ± 1.25 unit/mg protein) on the 1st and 2nd days after CPT-11 treatment, respectively, then partly recovered, but was still below the control activity on the 5th day. Lipid peroxide in the lung (Fig. 2) increased transiently, reaching 2.5 times ($P < 0.001$) the control level (2.04 ± 0.25 nmol/mg protein) on the 2nd day after CPT-11 administration, then decreased, but remained higher than the control for 5 days. GSHpx activity in the lung gradually decreased to 78.0% ($P < 0.01$) of the control (29.81 ± 0.82 unit/mg protein) on the 5th day. In the liver (Fig. 3), lipid peroxide increased transiently and was 1.9 times ($P < 0.01$) the control on

the 3rd day after CPT-11 administration. Thereafter, it recovered to the control level. There was no change in GSHpx activity in the liver of CPT-11-treated mice. The kidney showed a transient increase of lipid peroxide after CPT-11 injection, but the change was not significant. There was no change in GSHpx activity in the kidney.

Change in lipid peroxide level in rat tissues after CPT-11 administration (Fig. 4) In the heart, the lipid peroxide level gradually increased to 1.3 times ($P < 0.01$) the control level at 4 days after CPT-11 treatment. Lipid peroxide level in the bone marrow transiently increased after CPT-11 administration, reaching 1.6 times the control ($P < 0.05$) on the 2nd day, but reverted to the control level on the 4th day.

Effect of CPT-11 on NADPH-dependent microsomal lipid peroxidation of rat liver (*in vitro*) The lipid peroxide concentration of the control group with no CPT-11 or no adriamycin treatment increased about 8 times during incubation for 60 min after the addition of NADPH. In the presence of adriamycin (0.1 mM) and CPT-11 (2.95 mM, maximum added concentration), the lipid peroxide concentration increased 1.5 fold and 1.2 fold compared to the control, respectively.

Fig. 1. Time course of lipid peroxide level and GSHpx activity in the heart of mice after CPT-11 administration. Significant differences from the control value: a) $P < 0.001$ and b) $P < 0.01$.

Fig. 2. Time course of lipid peroxide level and GSHpx activity in the lung of mice after CPT-11 administration. Significant differences from the control value: a) $P < 0.001$, b) $P < 0.01$ and c) $P < 0.05$.

Fig. 3. Time course of lipid peroxide level and GSHpx activity in the liver of mice after CPT-11 administration. Significant difference from the control value: c) $P < 0.05$.

DISCUSSION

The LD_{50} (i.v.) of CPT-11 is 134 mg/kg in mice and 83.6 mg/kg in rats.²³⁾ The dosages of CPT-11 employed here were 100 mg/kg in mice and 60 mg/kg in rats, administered i.p.. The body weight of mice after CPT-11 administration at 100 mg/kg, i.p., transiently decreased on the 1st day and did not change thereafter. As the body weight in control mice increased by 10–15% in 5 days, it is considered that this dose of CPT-11 is toxic.

In mouse heart after CPT-11 administration, the lipid peroxide level was increased by 1.5 fold on the 5th day, compared to the control. It was also increased in the heart. GSHpx activity in mouse heart after CPT-11 treatment decreased to 64% of the control on the 2nd day, then partly recovered, but remained below the control level. After adriamycin administration, lipid peroxide level and GSHpx activity reached 2.6 times¹⁴⁾ and 54%¹⁵⁾ of the control level in the heart of mice, respectively. Therefore, the cardiotoxicity of CPT-11 should be weaker than that of adriamycin. However, combined chemotherapy including adriamycin (CAMP, CAP, MACC, etc.) is performed in lung carcinoma. Therefore, addition of CPT-11 in such combined chemotherapy

Fig. 4. Effect of CPT-11 on the lipid peroxide level in the heart and bone marrow of rats. Significant differences from the control value: b) $P < 0.01$ and c) $P < 0.05$.

might enhance the cardiotoxicity of adriamycin. Further studies are needed.

In the lung, the lipid peroxide level increased transiently on the 2nd day after CPT-11 administration, while GSHpx activity decreased to 78% of the control level on the 5th day after CPT-11 injection. Therefore, it is considered that the increment of the lipid peroxide level is related to the decrease in GSHpx activity. We have reported that tetrahydropyranlyadriamycin causes pulmototoxicity with an increment of lipid peroxide level.²⁴⁾ We suggested that the pulmototoxicity of CPT-11²⁵⁾ is severe because GSHpx activity in the lung did not recover to the control level, but this may be because the target tissue of CPT-11 is lung tumor. CPT-11 and SN-38, the active metabolite of CPT-11, are distributed more in the lung than in the heart, but the liver and kidney show the highest levels.²⁶⁾ Therefore, it is considered that the pulmototoxicity of CPT-11 is not directly related to the CPT-11 and SN-38 distributions, but may be an indirect effect, mediated by the inhibition of topoisomerase I activity.

In the liver, the lipid peroxide level increased transiently, but GSHpx activity did not change after CPT-11

treatment. As CPT-11 slightly increased NADPH-dependent microsomal lipid peroxidation *in vitro*, this effect may account for the increment of lipid peroxide level in the liver.

In rat bone marrow, the lipid peroxide level increased on the 2nd day after CPT-11 treatment and CPT-11 showed bone marrow toxicity. At this time, the numbers of white cells and platelets in blood were 62% and 25% of the control values, respectively. There has been no report on the determination of the lipid peroxide level in the bone marrow after administration of antitumor agents as an index of bone marrow suppression, but our results suggest that this might be a useful index, by analogy with the decrease in white cells and platelets after antitumor agent administration.

In conclusion, we suggest that the toxicity of CPT-11 may be related to the increment of lipid peroxide level in

the heart and lung. We also found an increase in the lipid peroxide level in the bone marrow after CPT-11 treatment. As CPT-11 has a weak effect on NADPH-dependent lipid peroxidation, it is considered that the cause of the increase in the lipid peroxide level is the CPT-11-induced decrease of GSHpx activity. CPT-11 also inhibits topoisomerase I activity and the biosynthesis of protein. In considering the use of CPT-11 in combination therapy, it may be necessary to take into account the interaction of these effects with those of the other agents to be used.

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