Injurious Effect of Buthionine Sulfoximine, an Inhibitor of Glutathione Biosynthesis, on the Lethality and Urotoxicity of Cyclophosphamide in Mice

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Accepted June 30, 1989

Abstract—The effect of buthionine sulfoximine (BSO), a glutathione biosynthesis inhibitor, on the acute lethal toxicity and urotoxicity induced by cyclophosphamide (CPA) was examined in mice. Pretreatment of mice with BSO (500 mg/kg, i.p.) 5 hr prior to CPA resulted in enhanced lethality and urotoxicity of CPA. In contrast, administration of cysteamine decreased the lethality and urotoxicity of CPA.

Cyclophosphamide (CPA), a widely used antitumor drug consisting of various alkylating agents, undergoes metabolic activation by hepatic mixed-function oxidases (1). Urotoxicity is a specific side-effect of alkylating agents characterized by the oxazaphosphorine (2), and CPA-induced urotoxic damage is due to the elimination of activated metabolites (particularly acrolein) (3, 4).

For various forms of antineoplastic intervention, glutathione metabolism helps to determine the degree of toxicity to the tumor and/or the host and the importance of glutathione in the toxicoology and metabolism of several toxins and drugs is well-known (5, 6). Recently, reduction of glutathione levels in tumor cells has been explored as a means of enhancing the cytotoxic effects of chemotherapeutic agents (7). Treatment with buthionine sulfoximine (BSO), a potent inhibitor of γ-glutamyl cysteine synthetase, has been reported to enhance the in vitro antitumor efficacy of a number of drugs including CPA, melphalan, mechlorethamine, nitrosourea, 6-thiopurine, adriamycin, daunomycin and mitomycin C (7, 8). In vivo sensitization of tumors by thioldepleting agents is accompanied by a decline in the glutathione content of normal tissues as well (8), but it has been reported that appropriate sulfhydryl-containing compounds provide effective protection against CPA-induced urotoxic effects without interfering with the chemotherapeutic activity of antitumor drugs (9). However, the effects of decreasing cellular thiols in vivo on either lethality or urotoxicity of CPA have not been extensively studied. The present study was designed to investigate whether glutathione depletion following BSO administration alters CPA lethality or the urotoxicity of CPA. BSO has fewer toxic side-effects (10, 11) and is therefore preferable for in vivo studies or for potential clinical use.

Male Std:ddY mice weighing 20–22 g from Shizuoka Laboratory Animal Center (Hamamatsu) were used. The animals were housed in a constant temperature and humidity environment (23±1 °C). Food and water were provided ad libitum. BSO was purchased from Sigma Chemical Co. (St. Louis, MO) and was administered in double-distilled, deionized water at a dose of 500 mg/kg 5 hr before the administration of CPA. CPA was obtained from Shionogi Pharmaceutical Co., Ltd. (Endoxan® for injection, Osaka) and was freshly dissolved in distilled water. Control animals were treated with identical volumes of solvent solution for each drug. All drugs were injected i.p. in a volume of 10 ml/kg body weight. In acute toxicity studies, mice were divided into experimental groups of 10 animals each and were injected with CPA at various doses. The mortality of the animals was observed for 21 days, during which the survivors were observed daily.
The assay of CPA-induced urinary bladder toxicity has been described previously (12), and the determination of renal glutathione levels were carried out according to the method described previously (12). A single dose of BSO (500 mg/kg, i.p.) depleted glutathione levels in the liver, kidney, heart, lung and stomach. The kidney and liver glutathione levels were maximally depleted within 5 hr. However, kidney glutathione remained depressed for 48 hr after the BSO injection, whereas liver glutathione had returned to the control value. The maximal reduction that was obtained in the kidney was 82%. The decrease of glutathione in the lung was 52% and those in the liver, heart and stomach were 70–80%. The effect of BSO on the acute lethal toxicity of CPA was studied at various dose levels. CPA doses were chosen in the range of toxic doses to allow determination of LD50 values. The potentiating effect of BSO on the lethal toxicity of CPA in mice is shown in Fig. 1. The lethality was observed for 21 days in those animal groups treated with CPA in doses of 450, 500, 550, 600 and 650 mg/kg and found to be 20%, 30%, 50%, 70% and 90%, respectively. In the BSO-treated mice, the lethality of CPA in smaller doses of 100, 150 and 200 mg/kg were 10%, 40% and 80%, respectively.

The LD50 of CPA was 520 mg/kg (i.p.). The pretreatment of BSO 5 hr prior to the administration of CPA increased the lethality of CPA dose-dependently. The LD50 of CPA was 150 mg/kg when combined with 500 mg/kg of BSO. This value is 3.4-fold as potent as the LD50 of CPA alone. Conversely, the administration of cysteamine both 5 min before and 30 min after CPA treatment afforded protection against the lethality of CPA. CPA caused a marked increase in bladder weight 48 hr after drug administration at doses of 200 mg/kg or higher. With the same treatment schedule as was effective against acute toxicity (Fig. 1), BSO enhanced the CPA-induced increase of bladder weight in mice at all doses of CPA tested (Fig. 2). Moreover, cysteamine prevented the CPA-induced increase of bladder weight in mice.

In the present study, we have demonstrated that pretreatment of BSO enhances the

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**Fig. 1.** Effect of buthionine sulfoximine on the lethality of cyclophosphamide in mice. Each group consisted of 10 animals. Cyclophosphamide (CPA) was administered to mice 5 hr after buthionine sulfoximine (BSO, 500 mg/kg, i.p.) injection. Cysteamine (100 mg/kg) was administered i.p. as two fractionated injections 5 min before and 30 min after CPA injection. Control animals received saline instead of BSO and cysteamine. Data are expressed as percent survival 21 days after CPA treatment. Symbols: Control, ○; CPA plus BSO, ●; CPA plus cysteamine, ▲.
lethality and urotoxicity of CPA. Our results, which show that exogenously administered cysteamine is an effective antidote for preventing the lethality and urotoxic lesions of CPA, are consistent with the suggestion that glutathione has an important role in the toxicity of this alkylating agent, presumably via alkylation of reactive metabolites. Like other thiol compounds, the nucleophilic structure of glutathione probably enables it to form an adjunct with electrophilic drug metabolites (6); thus, the uroprotective potential of cysteamine is not surprising.

One of the most important of the many functions of glutathione is the detoxication of reactive intermediates (5, 6). CPA undergoes biotransformation by cytochrome P-450 enzymes in the liver to form several electrophilic intermediates including phosphoramidate mustard (the active alkylating species) and acrolein (a bladder toxin devoid of antitumor activity). A range of reactive chemical species may be formed intracellularly either spontaneously or in reactions mediated by enzymes (5, 6). Glutathione can act either as a nucleophile with the formation of conjugates or as a reducing agent; when it participates in such a reaction, it is oxidized to its disulfide GSSG. Depletion of the content of glutathione in tissues has been shown to occur following exposure to many toxic agents, and the sequence of events may ultimately result in covalent binding of reactive metabolites to critical cellular macromolecules, with consequent lethality (13-15). Our results suggest that BSO should be used with extreme caution in experimental chemotherapy regimens using CPA where in significant dose, reductions will be mandatory.

References
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