The Deleterious Effect of Buthionine Sulfoximine, a Glutathione-Depleting Agent, on the Cisplatin Toxicity in Mice

Masaaki ISHIKAWA, Yoshio TAKAYANAGI and Ken-ichi SASAKI
Department of Pharmacology and Toxicology, Cancer Research Institute, Tohoku College of Pharmacy, Sendai 981, Japan
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Abstract—Pretreatment of buthionine sulfoximine (500 mg/kg, i.p., BSO), a potent glutathione-depleting agent, markedly increased the lethality and nephrotoxicity of cisplatin. These results suggest that a reactive electrophilic intermediate may be involved in the mechanism of cisplatin nephrotoxicity and moreover, that renal glutathione may play a protective role against cisplatin-induced nephrotoxicity.

Cisplatin (cis-platinum-II-diammine dichloride) is an antineoplastic drug with activity toward numerous animal and human tumors (1). However, its clinical use is limited by the dose-related cumulative impairment of renal tubular function (1).

Recently, depletion of cellular glutathione by buthionine sulfoximine (BSO), a potent inhibitor of r-glutamylcysteine synthetase (2), has been shown to sensitize tumor cells in vitro to certain chemotherapeutic agents such as cisplatin etc. (3). Such studies have led to a growing interest in the use of BSO as a chemosensitizer in cancer chemotherapy. However, glutathione may also play a similar role in the protection of normal tissues against the toxic effects of chemotherapeutic drugs. In this regard, BSO has been shown to potentiate the renal toxicity of 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (MeCCNU) and to result in a hepatotoxicity that is not ordinarily seen when MeCCNU is administered alone (4). Therefore, treatment strategies involving glutathione depletion should take into consideration the possible effect that glutathione depletion may have on the tissue sensitivity and target organ specificity of the antitumor drugs that are to be used in combination with compounds such as BSO. Our studies were designed to investigate whether glutathione depletion following BSO administration may alter the lethality or nephrotoxicity of cisplatin. BUN was used as a marker of nephrotoxicity.

Male mice of the ddY strain, weighing 15–17 g, were purchased from Japan SLC, Inc. (Hamamatsu) and housed as groups of 10 animals in plastic cages. They were kept in a room maintained at an ambient temperature of 23±1°C and given normal laboratory diet and tap water ad libitum. After reaching 19–20 g of body weight, they were used for the experiments. BSO and cisplatin were obtained from Sigma Chemical Co. (St. Louis, MO). BSO was dissolved in double-distilled, deionized water and administered i.p. at a dose of 500 mg/kg 5 hr before the administration of cisplatin. Control animals were treated with physiological saline. Cisplatin was dissolved in physiological saline at 0.5 mg/ml.

The determination of renal glutathione levels has been described previously (5). Acute lethality was calculated over a period of 7 days. Blood urea nitrogen (BUN) levels were measured colorimetrically following liberation of ammonia by urease using a diagnostic kit from Wako Pure Chemical Ind., Ltd. (Tokyo).

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. Kidney glutathione remained depressed for 48 hr, whereas liver glutathione had returned to the control value. Kidney glutathione levels were decreased to 37% of the control values (1.0±0.16 vs. 2.7±0.29 μM/g kidney weight, n=6 in each group). The decrease of gluta-
The effect of BSO pretreatment on cisplatin lethality in mice is shown in Fig. 1.

In this experiment, three groups of 10 mice were pretreated with cisplatin (18 mg/kg, i.p.) alone, or either BSO (500 mg/kg, i.p.) or physiological saline 5 hr before cisplatin (12 mg/kg, i.p.). The animals were monitored for 7 days, and deaths were recorded on a constant schedule. There were no deaths in the group of mice pretreated with physiological saline and then treated with cisplatin (12 mg/kg, i.p.). In the BSO-pretreated group, 9 of 10 mice were dead by the 5th day. The lethality induced by a higher dose of cisplatin (18 mg/kg, i.p.) during the entire 7-day period after injection was 80%, which was close to the percent of death observed with BSO plus cisplatin (12 mg/kg, i.p.). In addition, the lethality after 7 days was 20%, 45%, 90% and 100% in the groups treated with cisplatin alone at doses of 14, 16, 18 and 20 mg/kg, respectively. In the BSO-treated mice, the lethality was 30, 80 and 100%, which occurred in the groups treated with relatively smaller doses of cisplatin of 10, 12 and 14 mg/kg, respectively. Figure 2 shows that during the first 2 days after cis-

Fig. 1. Effect of buthionine sulfoximine on the cisplatin lethality in mice. A) Mice were administered saline plus cisplatin (12 or 18 mg/kg, i.p.) or cisplatin (12 mg/kg, i.p.) 5 hr after the injection of buthionine sulfoximine (BSO, 500 mg/kg, i.p.). ●, Cisplatin (12 mg/kg) alone; ○, BSO plus Cisplatin (12 mg/kg); △, Cisplatin (18 mg/kg) alone. B) Mice were administered with cisplatin 5 hr after the injection of BSO (500 mg/kg, i.p.). ●, Cisplatin alone; ○, Cisplatin plus BSO. Animals were observed for the following 7 days. Each point represents the percent lethality of 10–20 animals.

Fig. 2. Effect of buthionine sulfoximine on the cisplatin-induced nephrotoxicity in mice. Mice were administered saline plus cisplatin (12 or 18 mg/kg, i.p.), cisplatin (12 mg/kg, i.p.) at 5 hr after the injection of buthionine sulfoximine (BSO, 500 mg/kg, i.p.) or BSO plus saline. BUN was determined 48 hr after cisplatin (12 mg/kg) or cisplatin (12 mg/kg) plus BSO and 96 hr after cisplatin (18 mg/kg). Each column represents the mean value±S.E. obtained from 10 animals. Data were analyzed by Student’s t-test: *P<0.05 with respect to the control.
platin administration (12 mg/kg, i.p.) in animals pretreated with BSO 5 hr before cisplatin injection, there is a significant elevation of BUN levels, from 22 to 97 mg/dl, suggesting nephron functional damage. The groups of mice pretreated with physiological saline and then treated with BSO had no elevation of BUN values, but animals that were treated with cisplatin (18 mg/kg, i.p.) showed increased BUN values.

In this study, BSO was utilized to evaluate the effects of glutathione depletion at the time of cisplatin administration. Glutathione is known to be an intracellular detoxifying agent for inactivation and scavenger of free radical species (6). Therefore, it seems reasonable to study the effect of glutathione modulation on the lethality and nephrotoxicity of cisplatin. Considerable interest has been shown towards cisplatin-induced free radical production, particularly in relation to nephrotoxicity (7, 8). Because of this, we have studied the effects of glutathione depletion (5). The data presented in this study indicate that BSO pretreatment produced an increase in the acute lethal toxicity of cisplatin in the mouse, and this enhancement was reflected by an enhancement in renal toxicity. Recent studies have shown that BSO is useful as a specific agent for depleting tissue glutathione levels (9). In agreement with these studies, the data presented in this manuscript suggested that the potentiation of cisplatin toxicity caused by BSO pretreatment was due to lowered glutathione concentrations in the liver and kidney.

These results are in accord with those obtained using the general sulfhydryl trap (diethyl maleate), which has been shown in an in vivo system to increase the nephrotoxicity of cisplatin (10). Our data demonstrate that depletion of renal glutathione concentrations following BSO treatment compromised cellular defenses which resulted in enhanced cisplatin toxicity. The enhanced nephrotoxicity of cisplatin in glutathione-depleted mice is evidence that endogenous glutathione may also play a protective role in cisplatin nephrotoxicity. This suggests that renal glutathione concentration may be a critical factor in the expression of cisplatin nephrotoxicity.

However, these studies contrast with a study by Mayer et al. (11), who reported decreased cisplatin nephrotoxicity when BSO was used to reduce glutathione levels in rats. The apparent discrepancy between the data of Mayer’s group and our own findings regarding the effect of glutathione depletion on cisplatin nephrotoxicity may reflect the timing of BSO administration or species of animals used.

The mechanism by which BSO produces such an enhancement effect remains unknown. Glutathione plays a central role in the protection of cells against foreign compounds by acting as a reductant to remove peroxides and free radicals and by detoxifying reactive electrophiles via glutathione conjugate formation. Alteration in the endogenous levels of glutathione in rats treated with cisplatin has been reported, thus suggesting a possible in vivo interaction between cisplatin and glutathione (12). Sulfur-containing molecules are possible targets for nucleophilic attack by cisplatin (13). Indeed, several thiols have been reported to reduce the toxicity of cisplatin (14). This is consistent with an in vivo binding of platinum to sulfhydryl groups. Thus, enhancement of binding of cisplatin in the renal tubule could increase the drug induced renal damage. A similar toxication mechanism may operate in the enhancement of cisplatin nephrotoxicity by BSO.

It is clear that glutathione plays an important role in the cellular protection against a variety of antitumor drugs. Depletion of cellular glutathione by BSO has been shown to sensitize tumor cells to irradiation (15) and certain chemotherapeutic agents (3). Moreover, it has been demonstrated recently that the development of resistance of tumor cells to alkylating agents and radiation may in certain instances be related to increased cellular glutathione levels (15, 16). However, the potential usefulness of BSO or other thiol modulation drugs depends not only on a thorough understanding of possible drug interactions and/or host toxicities that may arise from combination therapies with various antitumors drugs, but also on an understanding of glutathione metabolism by the
tumor systems for which these therapies may be used. It is apparent that the influence of glutathione on cisplatin toxicity remains in question, and further studies will be required to elucidate the potential action of BSO.

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