Inhibition of Ifosfamide-Induced Urotoxicity by Disulfiram in Mice

Masaaki ISHIKAWA, Yoshio TAKAYANAGI and Ken-ichi SASAKI

Institute of Cancer Research, Tohoku College of Pharmacy, Sendai 983, Japan

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Abstract—The effect of disulfiram on the urotoxicity induced by ifosfamide was studied in mice. Ifosfamide administered intraperitoneally to mice caused a dose-related increase in bladder weight within 48 hr of treatment. Disulfiram prevented ifosfamide-induced bladder damage when administered orally within 1 hr of ifosfamide treatment. The results indicate that disulfiram is an effective protective agent against bladder damage caused by ifosfamide treatment.

Ifosfamide is one of the most widely used alkylating agents in cancer chemotherapy. Urotoxicity is a characteristic, specific side effect of alkylating agents with an oxazaphosphorine ring in the molecular structure (1). Acrolein has recently been found to be a metabolite of both ifosfamide and cyclophosphamide, and the investigators proposed that it contributes to the damage caused by the urotoxicity of these compounds (2, 3).

In view of the generally recognized difficulty of obtaining new drugs with increased efficacy, it is important to enhance the cytotoxicity of available antitumor agents against tumor cells by reducing toxic side effects. Protection against toxic effects could allow an increase in drug dose with potential therapeutic advantages.

The protective role that thiol compounds have against the toxic effects of alkylating agents has long been recognized (4).

Recently, the parenteral administration of certain sulfhydryl-containing compounds such as N-acetylcysteine (5), 2-mercaptoethane sulfonate (6), dimercaptosuccinic acid (7), and disulfiram (8) have been reported to prevent hemorrhagic cystitis produced by cyclophosphamide. Many of the histological lesions caused by cyclophosphamide are similar to those observed in ifosfamide-induced hemorrhagic cystitis (2, 3), and disulfiram has also been reported to decrease the toxicity of several alkylating agents (9, 10) and to inhibit the carcinogenicity of various N-nitroso compounds (9, 11).

This study was undertaken to determine

whether oral administration of disulfiram influences bladder damage induced by ifosfamide treatment.

Male ddY mice, 5 weeks old and weighing 22–24 g, obtained from the Shizuoka Animal Center (Hamamatsu, Japan) were used. They were given standard rodent pellet and water ad libitum and housed at a constant temperature and humidity environment in the university vivarium. Disulfiram was obtained from Tanabe Pharmaceutical Co., Ltd., (Osaka, Japan) in the powder form. The powder was then suspended in 0.8% Tween 80-physiological saline and sonicated for 10 min (Braunsonic). Ifosfamide was obtained from Shionogi Pharmaceutical Co., Ltd. (Ifomide® for injection, Osaka, Japan). In all studies, ifosfamide was administered intraperitoneally, and disulfiram was administered orally in a volume of 10 ml/kg body weight. The assay of bladder toxicity, based on the drug-induced increase of bladder weight, was essentially similar to that of Brock et al. (1). Briefly, mice were given various doses of ifosfamide, i.p., and killed by cervical dislocation 48 hr later. The bladders were excised immediately, blotted and weighed to obtain the relative wet weight to body weight for each animal (mg of bladder weight per 100 g of body weight). To correlate bladder weight changes with ifosfamide-induced bladder damage, bladders were fixed in 10% neutral phosphate-buffered formalin and prepared for histological study. Determination of statistically significant differences between treatment groups was

calculated using Student's *t*-test.

The effect of disulfiram on the urotoxicity of ifosfamide was studied at various dose levels of both the antitumor agent and thiol compound. Ifosfamide caused a marked increase in bladder weight measured 48 hr after drug administration at 100 mg/kg or higher in a dose-related manner (Fig. 1). The effect of disulfiram on the ifosfamide-induced changes in bladder weight was studied at various dose levels of ifosfamide. When disulfiram was administered simultaneously with ifosfamide by the oral route, as can be seen in Fig. 1, disulfiram effectively prevented the ifosfamide-induced increase in bladder weight in mice at each dose of ifosfamide. The uroprotective effect was further substantiated by histological study of bladders removed from mice treated with ifosfamide (200 mg/kg), ifosfamide (200 mg/kg) plus disulfiram (200 mg/kg), or saline solution. Ifosfamide administration resulted in edema of the lamina propria and muscularis, mild inflammatory reaction, and disruption of the epithelial lining. By contrast, mice given disulfiram or disulfiram plus

ifosfamide had no histological evidence of bladder damage.

Since the protective efficacy of thiol compounds on the bladder is critically dependent on the timing of administration (8), further experiments were designed to determine whether the protective effect of disulfiram is dependent on administration schedule or not. When given simultaneously with ifosfamide, disulfiram prevented bladder weight increase at doses lower than 200 mg/kg (Fig. 1). In the subsequent studies, therefore, 200 mg/kg of disulfiram was used. To determine the most effective schedule for the inhibition of ifosfamide-induced increase in bladder weight, disulfiram (200 mg/kg) was administered at various times before or after ifosfamide. These results are shown in Fig. 2. An oral administration of disulfiram between 60 min before and 60 min after injection of ifosfamide was effective. The protective efficacy of disulfiram was diminished in other periods of time. When administered 120 min before or 120 min after injection of ifosfamide, disulfiram did not protect against the increase of bladder weight.

Fig. 1. Protection against ifosfamide-induced bladder toxicity by disulfiram in mice. Mice were given various doses of ifosfamide (IFX, i.p.) simultaneously with various doses of disulfiram (DSF, p.o.), and 48 hr later, bladder weights were measured. Bladder weights were normalized to body weight (mg bladder weight per 100 g of body weight). Each value is presented as the mean ($\pm$ S.E.) of 8–10 animals. ^{a)}values significantly different from the control ($P < 0.05$). ^{b)}significantly different from ifosfamide alone ($P < 0.05$).

Fig. 2. Influence of treatment schedule on the protective effect of disulfiram in ifosfamide-induced bladder toxicity in mice. Mice were given ifosfamide (200 mg/kg, i.p.) and disulfiram (200 mg/kg, p.o.) at a selected time with respect to ifosfamide. Bladder weights were measured 48 hr after ifosfamide administration. Values are the means ($\pm$ S.E.) for 8–10 animals. ^{a)}values significantly different from the control ($P < 0.05$). ^{b)}values significantly different from ifosfamide alone ($P < 0.05$).

Unlike most other alkylating agents, ifosfamide requires metabolic activation to attain significant cytotoxic and alkylating activity. Indeed, the metabolic products of ifosfamide, including acrolein which is characterized by weak cytotoxic properties, and chloroacetaldehyde have been implicated in this characteristic toxic effect (1, 3), which is a limiting factor in the therapeutic use of the drug, particularly in high-dose chemotherapy regimens.

Because of this dose-limiting toxicity of ifosfamide and related compounds, a great deal of effort has been expended in an attempt to develop a drug which is as effective as ifosfamide and lacks urotoxic potential.

Appropriate sulfhydryl-containing compounds have been reported to provide effective protection against cyclophosphamide-induced urotoxic effects, probably through deactivation of toxic metabolites (1, 5). In the mechanism of the protective action of these thiol compounds, a central role of endogenous glutathione has been generally recognized (12). In particular, a preferential interaction of the endogenous glutathione with the electrophilic metabolite, acrolein, has been proposed as a mechanism for protection against some toxic effects of cyclophosphamide (12). Our results, which show that simultaneously administered disulfiram is effective in preventing ifosfamide-induced

urinary bladder changes in mice are consistent with the suggestion that endogenous 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 would enable it to form an adduct with electrophilic drug metabolites (13). Thus, the uroprotective potential of disulfiram is not surprising. Further, unlike the other uroprotective agents, disulfiram demonstrated uroprotection when administered by the oral route. Since disulfiram is currently in clinical use and has few undesirable effects, its use in patients at high risk for ifosfamide-induced bladder injury would warrant investigation.

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