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The effect of disulfiram on cyclophosphamide-mediated myeloid toxicity

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Summary. We have previously shown that disulfiram (DSF) blocks the urotoxicity of cyclophosphamide (CYT) in mice and increases the oncolytic effect of CYT in the L1210 murine leukemia. However, mice treated with CYT and DSF appeared to have longer-lasting neutropenia than animals treated with CYT alone. To determine whether DSF uroprotection of CYT-treated mice was associated with increased myeloid toxicity, we examined the effects of DSF plus CYT treatment on the bone marrow granulocyte/macrophage progenitor cell (GM-CFC). Marrow cellularity and GM-CFC numbers were analyzed at 1, 2 and 3 days after injection of CYT (62.5 or 125 mg/kg) or CYT plus DSF (200 mg/kg). CYT alone caused a decrease in total marrow cellularity varying from 20% to 50% of control. Animals given CYT plus DSF had a somewhat greater decrease in total marrow cellularity than those treated with CYT alone. However, in mice treated with CYT plus DSF, the GM-CFC were relatively well preserved and the recovery of the GM-CFC was not prolonged by DSF. It appears from these studies that the acute toxic effect of CYT on the granulocyte/macrophage progenitor cells is not enhanced by DSF.

Introduction

Effective treatment with cyclophosphamide (CYT) is occasionally limited by severe urotoxic complications, especially hemorrhagic cystitis [12]. We have recently shown that disulfiram (tetraethylthiuram disulfide, DSF) effectively protects the urinary bladder when given to mice prior to or just following CYT [9, 10]. Furthermore, DSF enhanced the oncolytic effect of CYT against L1210 murine leukemia [10]. However, while there was a comparable degree of leukopenia in mice treated with CYT and mice treated with CYT and DSF, the duration of leukopenia was slightly longer in mice given CYT and DSF. Whereas complete recovery was evident 9 days after CYT administration, mice that received CYT and DSF did not recover completely until 11 days after treatment. The results of these previous studies suggested that DSF might actually enhance CYT-mediated cytotoxicity of both tumor cells and host hematopoietic elements. Since this idea was based primarily on observations of peripheral leukocyte number, we felt further evaluation of the effect of DSF on CYT myelotoxicity was necessary. We therefore examined the effect of DSF and/or CYT on the femoral bone marrow granulocyte/macrophage progenitor cell compartment. It appears that overall marrow cellularity is reduced in animals treated with the DSF/CYT combination compared to those given CYT alone, but this reduction is transient and *not* associated with augmented toxicity to the myeloid progenitor cell compartment.

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Materials and methods

Mice. Male albino CD mice 20–25 g in weight were purchased from Canadian Breeders Laboratory, Montreal, Quebec. Animals were housed in a central animal care facility which maintains an environment of controlled temperature, relative humidity and 12-h light/dark cycles (06.00–18.00 h light). Food and water were available ad libitum.

Drugs. Clinical formulation Cytoxan (cyclophosphamide; CYT was purchased from Mead Johnson Co., Evansville, Ind, and was injected IP at doses of 62.5 or 125 mg/kg. Disulfiram (tetraethylthiram disulfide) was obtained from Sigma Chemical Corporation, St. Louis, Mo, and was administered at a dose of 200 mg/kg via gavage 30 min prior to cyclophosphamide injection.

GM-CFC culture technique. We used a modification of the technique of Bradley and Metcalf for in vitro quantitative clonal culture of the granulocyte/macrophage progenitor cell (GM-CFC) [3, 8]. Cultures were plated in 35-mm Falcon plastic petri dishes containing 1 ml McCoy's medium supplemented with 15% fetal calf serum (Grand Island Biologic Co.) and 3% Difco Bacto agar containing 2000 units each of penicillin and streptomycin mixture (Microbiologic Associates, Inc., Bethesda, Md). Each of three replicate plates contained 50 000 nucleated femoral marrow cells per mouse and 0.02 ml postendotoxin serum, a supramaximal dose of colony-stimulating factor (CSF). After mixing, the plates were allowed to gel at room temperature for 20 min and were incubated for 7 days at 37 °C in a humidified 10% CO_2–air atmosphere. Colonies were scored with a dissecting microscope at a magnification of 10×, and aggregates containing 50 or more cells were scored as colonies.

Data analysis. The change in bone marrow cellularity (i.e., number of nucleated bone marrow cells/femur) induced by the drug treatment is calculated by comparing the cell count in the experimental group with that of the sham treatment control group. Similarly, bone marrow progenitor cells in the experimental groups on each day after treatment were compared to the sham treatment groups simultaneously analyzed. The results for each assessment (marrow cellularity and progenitor cell number) are therefore expressed as percentages of control (mean values ± standard error) Student's *t*-test was used to determine the significance of any differences between individual treatment groups.

Results

In an initial series of experiments, DSF was found to have no effect on either the total marrow cellularity or the number of marrow GM-CFC on days 1, 2, or 3 after oral administration of 200 mg/kg (Table 1).

To determine whether DSF would in some way alter the marrow toxicity of CYT, mice were administered a single IP injection of either 62.5 or 125 mg/kg CYT, followed 30 min later by an oral dose of DSF (200 mg/kg) or 0.9% NaCl. Total marrow cellularity and GM-CFC content were measured 24, 48 and 72 h after drug treatment. As depicted in Fig. 1, the total marrow cellularity was significantly more depressed in mice administered 62.5 mg/kg CYT plus DSF than in mice given CYT alone at 24 and 48 h post-treatment, but was no different by 72 h. Similar, but less consistent, results were obtained in mice given 125 mg/kg CYT, with a suggestion that DSF enhanced the CYT-induced decrease in marrow cellularity.

In contrast, DSF appeared not to augment the toxic effect of CYT on marrow GM-CFC (Fig. 2). In fact, the survival of GM-CFC in mice given CYT (62.5 mg/kg) plus DSF was significantly greater than that of mice given CYT alone when measured 24 h after drug treatment. The CYT/DSF-treated mice had a maximal GM-CFC overshoot on day 2 and were returning to control values by day 3. Recovery (as indicated by peak overshoot) in mice given CYT alone did not occur until day 3, suggesting a delay in marrow recovery in these mice compared with mice given DSF in combination with the lower dose of CYT. Although this unexpected marrow protection was not observed in mice given DSF and the higher CYT dose, the combination was no more toxic than CYT alone (Fig. 2).

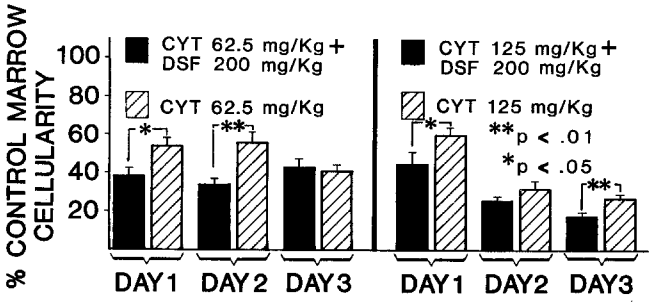

Fig. 1. Total femoral bone marrow cellularity on days 1, 2, and 3 after administration of CYT or DSF and CYT. The change in marrow cellularity induced by cytoxan with or without disulfiram is represented by comparison with the sham-treated controls and reported as percentage of control ± SEM. (Eight animals per group)

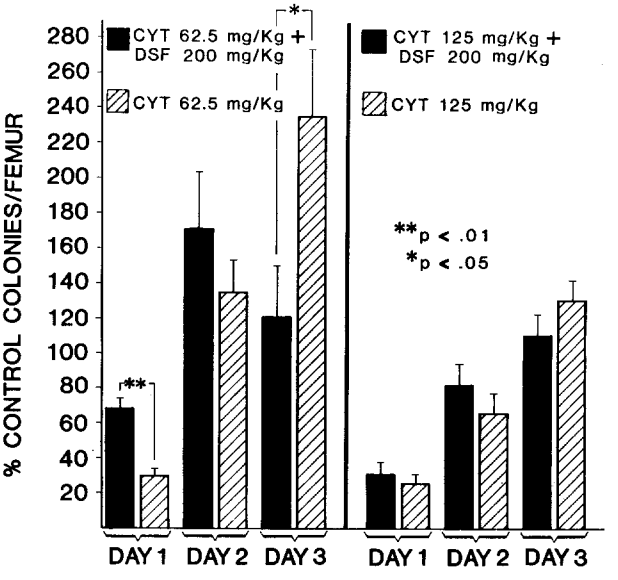

Fig. 2. Progenitor cell (GM-CFC) growth on days 1, 2, and 3 after administration of CYT or DSF and CYT. The change in GM-CFC induced by cytoxan with or without DSF is represented by comparison with the sham-treated controls and reported as percentage of control ± SEM. (Eight animals per group)

Discussion

To further evaluate possible DSF augmentation of CYT-induced myelotoxicity, we designed the studies reported above. Marrow cellularity after combined DSF/CYT treatment appeared to be reduced significantly compared with marrow from animals receiving CYT alone. This finding correlates with the greater duration of leukopenia observed in our earlier study, where we used comparable doses of CYT and DSF [10]. Nevertheless, the number of

Table 1. The effect of disulfiram on the bone marrow

	Saline			Disulfiram[c]		
Day[a]	*n*[b]	Cells/femur × 10^6	GM-CFC/femur	N	Cells/femur × 10^6	GM-CFC/femur
1	8	184.9 ±5.9	23488 ±4722	8	193.5 ±10	22858 ±2798
2	8	190 ±19.6	21655 ±2961	8	210.9 ±16.7	25974 ±3232
3	8	213 ±11.6	30413 ±3522	8	174.3 ±15.7	25305 ±4504

[a] Days following injection of saline or disulfiram
[b] Number of animals per group
[c] Disulfiram 200 mg/kg via gavage

myeloid progenitor cells was not reduced to a greater extent in animals treated with both drugs. In fact, for the lower CYT dose it was apparent that the total number of myeloid progenitor cells present 24 h after the CYT dose (nadir) was lower in animals receiving CYT alone. At the higher CYT dose, comparable reductions were observed. The GM-CFC marrow recovery occurred earlier in the large-dose DSF/CYT animals than in the CYT-alone animals. However, the ultimate extent of recovery was similar. There were no differences in the time or extent of GM-CFC recovery for the higher dose of CYT.

It appears from these studies that the acute myeloid injury and marrow recovery following CYT are not altered by DSF. If DSF does mediate myeloid toxicity it must presumably do so at a more mature cellular stage, beyond the progenitor cell level. The decrease in total marrow cellularity is certainly due in large part to cytotoxicity, but there may also be a component of cell migration. We have not tested the possibility that other marrow cells, such as lymphocytes or erythroid precursors, may be reduced by the DSF/CYT combination and account for the reduced cellularity we observed.

DSF is known to be rapidly hydrolyzed to sulfhydryl-containing metabolites [7]. Sulfhydryl-containing compounds have been shown to form adducts with the urotoxic metabolite of CYT, acrolein [4]. Initially, we speculated that a similar mechanism (DSF-acrolein adduct formation) was the mechanism of DSF-mediated uroprotection. DSF has also been reported to inhibit a number of enzyme systems, including certain of the mixed function oxidase system enzymes, aldehyde oxidase, and aldehyde dehydrogenase. These enzymes are critical in the activation and metabolism of CYT. The impression that significant inhibition of the mixed function oxidase system (which is required for CYT activation) does not occur, is supported by the enhanced rather than the reduced CYT oncolytic effect in the L1210 leukemia in animals co-treated with DSF. It is possible, however, that inhibition of aldehyde dehydrogenase, an enzyme system responsible for the production of the noncytotoxic metabolites, carboxyphosphamide, and 4-ketocyclophosphamide, might result in an increased production of phosphoramide mustard, the proposed alkylating moiety of CYT. If this were the case, while this could lead to a heightened oncolytic effect of CYT, it would also be associated with increased marrow toxicity. The lack of such a corresponding increased myeloid toxicity suggests that the effects observed following co-treatment with CYT and DSF are more complex than either simple adduct formation or aldehyde dehydrogenase inhibition and may be related to a combination of these events.

Other compounds are currently under investigation as uroprotectants for CYT-treated patients. *N*-Acetylcysteine [1, 11] and 2-mercaptoethane sulfonate [5] have both proven effective in preclinical studies and are currently being investigated in clinical trials. We have completed a preliminary clinical trials (phase I) to assess DSF toxicity in CYT-treated patients [6]. Peripheral white blood cell nadirs were no lower or more prolonged when CYT was co-administered with DSF. There was a suggestion of more nausea and vomiting with the combined therapy, but this was not striking.

A logical extension of these preclinical and early clinical studies to assess the efficacy of DSF uroprotection would be a trial in human bone marrow transplant recipients during the cytoreductive preparative stages prior to bone marrow infusion [2]. High-dose (60 mg/kg) CYT is frequently used in this setting with total-body irradiation, and despite hydration and vigorous supportive measures hemorrhagic cystitis occasionally occurs. Hematuria is common and particularly dangerous in the thrombocytopenic patient. Successful uroprotection by a noninvasive and nontoxic means would represent an advance in the management of these patients. Without identifiable augmentation of CYT host toxicity, including myelotoxicity, we propose that DSF may prove to be a clinically useful uroprotectant in CYT-treated patients.

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