Combinations of Mesna with Cyclophosphamide or Adriamycin in the Treatment of Mice with Tumors

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ABSTRACT

Following therapeutic administration, cyclophosphamide and Adriamycin are biotransformed to reactive metabolites, some of which are responsible for undesirable systemic toxicities of these chemicals, whereas others are responsible for their chemotherapeutic effectiveness. Microsomal mixed function oxidases activate cyclophosphamide to produce phosphoramide mustard and acrolein, while cytochrome reductase and xanthine oxidase are capable of transforming Adriamycin and forming free radicals. These reactive metabolites produce unwanted toxic side effects; however, their action may be partially ameliorated by the concomitant administration of thiols. In this study we evaluated the therapeutic activity of combinations of mesna (2-mercaptoethanesulfonate) with cyclophosphamide or Adriamycin in mice with a variety of transplantable tumors (L1210 and P-388 leukemia, Lewis lung and colon 26 carcinoma, B16 melanoma, and M5076 sarcoma). In all cases the administration of mesna prior to cyclophosphamide or Adriamycin treatment did not reduce the antitumor effectiveness of these agents and in some instances (C57BL/6 mice with B16 melanoma or M5076 sarcoma) small improvements were observed. Therefore, the addition of thiols, to reduce effectively the buildup of toxic metabolites of cyclophosphamide or Adriamycin may result in the improved therapeutic effectiveness for these agents in the treatment of cancer.

INTRODUCTION

Cyclophosphamide (2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide) and Adriamycin are important anticancer drugs used in the treatment of a variety of human malignancies (1, 2). In addition, these agents have been observed to have differential immunosuppressive activities (3) with cyclophosphamide finding use in organ transplantation procedures (4) and for the treatment of some autoimmune diseases (5).

Both Adriamycin and cyclophosphamide are transformed metabolically into a number of different pharmacologically and toxicologically active metabolites. Adriamycin acts as an electron acceptor and in the presence of flavoenzymes (cytochrome reductases and xanthine oxidase) may generate either oxygenic or drug-radicals, depending on the availability of oxygen. The anti-tumor activity of Adriamycin, and its immunomodulatory action and cytotoxicity may be related directly to the formation of specific metabolites. Administration of other drugs, such as allopurinol, an inhibitor of xanthine oxidase (6), or thiols, may affect the biotransformation of Adriamycin and perhaps may result in better therapeutic activity and reduced toxicity. The actions of cyclophosphamide also may be modulated with similar compounds. The pharmacological actions of cyclophosphamide are absolutely dependent upon metabolic activation (7); per se, it is inactive. Its carcinostatic activity depends on its metabolism by the hepatic microsomal mixed function oxidase system leading to the formation of phosphoramide mustard (8). However, the metabolism of cyclophosphamide also leads to the production of acrolein, a toxic metabolite responsible for the depression of this enzyme system via inactivation of hepatic cytochrome P-450 (9). Further, acrolein damages host bladder epithelium leading to hematuria which in many cases is the dose-limiting toxicity for the administration of cyclophosphamide (10–12). Biochemical studies have demonstrated that the addition of thiols (glutathione, cysteine, or N-acetylcysteine) directly to rat liver microsomes in vitro can prevent acrolein-induced decreases in cytochrome P-450 (9, 10). Additionally, it has been observed that coadministration of N-acetylcysteine with isophosphamide (13) or mesna with cyclophosphamide (10–14) can ameliorate some cyclophosphamide host toxicities attributed to acrolein. Preliminary therapeutic studies on the combination of cyclophosphamide with mesna in rats with Walker carcinoma demonstrated no decrease in the antitumor activity of cyclophosphamide by the addition of mesna (15). This report extends those studies and describes the therapeutic activity of combinations of mesna with cyclophosphamide or Adriamycin in mice with a number of different transplantable tumors.

MATERIALS AND METHODS

Drugs. Cyclophosphamide was obtained from the Drug Development Branch, National Cancer Institute, Bethesda, MD. Adriamycin was provided by Adria Laboratories and mesna was a gift from Dr. Brock (12). All drugs were dissolved in saline and injected i.p. into mice, or 0.2-ml aliquots.

Mice and Tumors. Female C57BL/6 mice, 7–10 weeks old, weighing 19–20 g, were obtained from the West Seneca animal facilities of this Institute. Fifty-mg pieces of nonnecrotic, viable Lewis lung carcinoma, M5076 sarcoma, or B16 melanoma, originally provided to this laboratory by the National Cancer Institute, DCT Tumor Repository, Frederick Cancer Research Facility, Frederick, MD, were transplanted s.c. by trochar implant in the abdominal flank. Three days later tumors were palpable and drugs were administered i.p. Mesna (250 mg/kg) administration when given in combination with cyclophosphamide (250 mg/kg) or Adriamycin (7.5 mg/kg), preceded those agents by 20 min.

Tumor growth was monitored every 3 days by caliper measurements of 2 perpendicular diameters, and tumor mass (cm³) was estimated algebraically by using the equation

\[0.4 \times \text{long axis} \times \text{short axis}^2\]

Data processing was performed with software prepared for an Apple microcomputer (16, 17). Animal weight was measured at the same time. Mouse survival was checked daily for at least 60 days. Statistical comparisons were performed as described in Tables 1–4.

Colon 26 carcinoma was transplanted in 8- to 10-week-old, female BALB/c mice (20–25 g) obtained from the breeding colony of this Institute. A 50-mg piece of colon 26 carcinoma, originally obtained from Dr. T. Corbett, Michigan Cancer Foundation, Detroit, MI, was transplanted s.c. by trochar into the abdominal flank. All other procedures are similar to those described above for the other solid tumors transplanted in C57BL/6 mice.

Leukemic L1210 or P-388 cells were transplanted i.p. into female DBA/2N mice, weighing 19–20 g and 8 to 10 weeks old obtained from the NCI-Frederick Cancer Research Facility. Animal survival was monitored daily and death weights were recorded. Drug treatments (i.p. injections) were administered on day 2 using dosages outlined previously.
RESULTS

Previously we reported that the administration of thiols either i.p. or i.v. followed by i.p. cyclophosphamide afforded a degree of protection against urotoxicity, inhibition of microsomal mixed function oxidase activities, and the depression of cytochrome P-450 content. Furthermore, we noted that i.p. mesna followed by i.p. cyclophosphamide did not interfere with the chemotherapeutic activity of cyclophosphamide against Walker 256 carcinosarcoma in rats. In this study, we extended our examination of this combination of agents as an antitumor regimen in mice.

The administration of cyclophosphamide on day 2 (Table 1), with or without mesna, to DBA/2N leukemic mice resulted in significant increases in life span (% ILS). Cyclophosphamide alone caused a small decrease in animal weight and a 130% ILS in DBA/2N mice with L1210 leukemia. In mice with P-388 leukemia, cyclophosphamide also caused a small loss in body weight (from 20 to 18.2 g) and only a 12% ILS.

Mesna treatment alone on day 2 resulted in no apparent effect on leukemic mouse weight or lifespan. The combination of mesna with cyclophosphamide again resulted in a small loss in animal weight but an even larger (144% ILS) increase in L1210 leukemic mouse life span. In mice with P-388 leukemia the combination of mesna with cyclophosphamide marginally was effective, evoking only a 10% ILS with a small weight loss (20 to 17.4 g). However, it is apparent from these studies that the addition of mesna does not reduce the therapeutic effectiveness of cyclophosphamide.

The efficacy of Adriamycin in the treatment of murine leukemias also was not compromised by the addition of mesna (Table 2). Adriamycin administered to L1210 leukemic mice increased mean life span from 6.6 to 10.2 days with a 55% ILS. In mice with P-388 leukemia, an 80% ILS was achieved. Mesna alone had no measurable effect and in combination with Adriamycin did not reduce its therapeutic effectiveness. In fact, in mice with P-388 leukemia a 98% increase in mean life span (from 9.4 to 18.6 days) was observed (Table 2).

In female C57BL/6 mice transplanted s.c. with a variety of solid tumors cyclophosphamide caused a significant reduction in tumor volume with a corresponding increase in life span (Table 3). Mice with B16 melanoma given cyclophosphamide reduced tumor size by 53.5 days (15 to 29% ILS). Additionally, mice with P-388 leukemia treated with the addition of mesna had an increased mouse survival (to 77% ILS, with one long term survivor being free of tumor). Adriamycin also increased mean survival time 56%, i.e., from 28.5 to 44.6 days (Table 3). Mesna alone had no measurable effect; however, in combination with cyclophosphamide, a larger reduction in tumor volume and an increased mouse survival (to 77% ILS, with one long term survivor being free of tumor) was noted.

In female C57BL/6 mice implanted s.c. with M5076 sarcoma, the addition of mesna treatment to cyclophosphamide increased mean life span from 47.7 days (cyclophosphamide alone) to 53.5 days (15 to 29% ILS). Additionally, median tumor size was significantly reduced by the addition of mesna.
with half-maximal median tumor size being reached on day 55 rather than day 49 (for cyclophosphamide treatment alone). Mesna alone appeared to reduce tumor size, however, this difference was not statistically significant (Table 3).

Female C57BL/6 mice transplanted s.c. with Lewis lung carcinoma responded most dramatically to cyclophosphamide administration (Table 3). Of 20 mice with Lewis lung carcinoma, 19 were cured with either cyclophosphamide alone (10 of 10) or with the combination of mesna and cyclophosphamide (9 of 10). The one mouse that died at day 36 in the group receiving the combination was tumor free at day 26 and was essentially cured. Mesna alone had no effect.

Colon 26 carcinoma transplanted in female BALB/c mice also responded to cyclophosphamide administration (Table 4). Cyclophosphamide alone increased life span by 196% with a concomitant decrease in tumor size (half-maximal tumor volume was achieved on day 33 versus day 16, untreated). The additional mesna pretreatment increased the therapeutic efficacy of cyclophosphamide, resulting in a 226% ILS; the administration of mesna alone had no effect.

DISCUSSION

The administration of cyclophosphamide and other anticancer drugs can lead to a depression of the hepatic mixed function oxidase system (18) via the formation of bioreactive metabolites. Acrolein, a by-product of the hepatic activation of cyclophosphamide to phosphoramide mustard, has been shown to be the responsible agent for the depression of cytochrome P-450 (9). It is the cause of the urotoxicity of cyclophosphamide which in many cases is dose limiting. Similarly, Adriamycin can be metabolized to bioreactive products including free radicals capable of causing unwanted cellular damage such as lipid peroxidation.

The administration of additional agents with cyclophosphamide or Adriamycin, to lessen these toxic side effects resulting from the generation of bioreactive metabolites, may result in improved therapeutic regimens for these active, antitumor agents. Thios given prior to cyclophosphamide have been shown to protect cytochrome P-450-dependent enzyme activities and to decrease acrolein-induced urotoxicity in rats (10).

Similarly, the coadministration of allopurinol, a xanthine oxidase inhibitor, with Adriamycin has been demonstrated to decrease the formation of 7-deoxyxaglycine metabolite of Adriamycin and enhance its antitumor activity (6).

In earlier reports (9, 10, 19–21), it was demonstrated that cyclophosphamide-induced depression of hepatic glutathione, its decrease in microsomal enzyme activities, and its urotoxicity were due to an interaction between the cyclophosphamide metabolite, acrolein, and free and tissue-bound thiols. In one of these reports (19), comparison of hepatic glutathione-depleting efficacies of cyclophosphamide, its metabolites and analogues demonstrated that acrolein was at least one order of magnitude more reactive toward glutathione than was the chemotherapeutically active cyclophosphamide metabolite, phosphoramide mustard. These results suggested a possibility of blocking acrolein-related toxicities without interference of the chemotherapeutic activity of cyclophosphamide by providing exogenous thiols, such as N-acetylcysteine or mesna. Subsequently, we reported that mesna does not interfere with the chemotherapeutic activity of cyclophosphamide against Walker 256 carcinoma implanted in rats (15). Mesna combined with cyclophosphamide also has been shown to block or ameliorate some of the undesirable cyclophosphamide toxicities, such as depression of hepatic mixed function oxidase system and urotoxicity (10, 14).

In the present report we have extended chemotherapy studies with the combination of mesna plus cyclophosphamide to include several mouse tumors in order to test the hypothesis that mesna, known to prevent or reduce some of the undesirable toxicities of cyclophosphamide, will not interfere with the chemotherapeutic activity of cyclophosphamide.

The results of these studies demonstrated negligible effects of the combination of mesna on the antitumor activity of cyclophosphamide. In certain instances there appeared to be an enhanced therapeutic effectiveness of the combination. Based on the percentage of ILS, the coadministration of mesna prior to cyclophosphamide increased the life span in mice with L1210 leukemia, colon 26 carcinoma, B16 melanoma, and M5076 sarcoma. Additionally, mesna had no detrimental effects on the antitumor activity of Adriamycin and the combination of these agents resulted in a better ILS in mice with P-388 leukemia. Brock et al. (14) have also reported on the inability of mesna to interfere with the chemotherapeutic activity of oxazaphorine anticancer agents.

Overall, the coadministration of mesna with Adriamycin or cyclophosphamide, to decrease toxic side effects of these agents, resulted in no apparent reduction in their antitumor effectiveness.

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REFERENCES


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