Interaction of Cimetidine but not Ranitidine with Cyclophosphamide in Mice

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ABSTRACT

A series of experiments in DBA/2J mice evaluated the biological and pharmacokinetic interactions of the alkylating agent cyclophosphamide (CTX) and the histamine-H2 antagonists cimetidine (CMT) and ranitidine (RNT). Doses were adjusted to approximate human dose levels: 100 mg/kg for CMT; and 25 mg/kg for RNT. CTX reduced the survival of normal (bone marrow stem cell) colony forming units in a dose dependent fashion. CMT, given 5 or 30 min before CTX (200 mg/kg), significantly increased the survival of leukemia bearing mice, as well as the elimination half-life and plasma area under the curve of total alkylating metabolites of CTX. RNT did not significantly alter CTX antileukemic activity, pharmacokinetics, or toxicity to normal bone marrow stem cells. These results suggest caution in the use of CMT in patients being treated with CTX in order to avoid the possibility of exaggerated CTX toxicities. RNT may be a safer histamine-H2 antagonist to use with CTX if a histamine-H2 antagonist is clinically indicated.

INTRODUCTION

A number of anticancer drugs are actively metabolized by microsomal enzymes to chemical species with altered pharmacological properties. Some of the well characterized drugs in this group are metabolized to species more active than the parent compound. This is the case with the DNA alkylators CTX (1), dacarbazine (2), procarbazine (3, 4), and hexamethylmelamine (5). Conversely, a number of anticancer drugs are metabolized to inactive (noncytotoxic) species which are excreted in the urine or bile. Agents in this category include the nitrosoureas, carmustine and lomustine (6), the DNA intercalating anthracycline antibiotic doxorubicin (Adriamycin) (7) and, also paradoxically, CTX (1, 8). CTX is metabolized to a series of compounds which are active in vitro but not in vivo (9). Some of these metabolites are more active in vivo than CTX (10). CTX is also metabolized to species more active than the parent compound. This is the case with the DNA alkylators CTX (1), dacarbazine (2), procarbazine (3, 4), and hexamethylmelamine (5). Conversely, a number of anticancer drugs are metabolized to inactive (noncytotoxic) species which are excreted in the urine or bile. Agents in this category include the nitrosoureas, carmustine and lomustine (6), the DNA intercalating anthracycline antibiotic doxorubicin (Adriamycin) (7) and, also paradoxically, CTX (1, 8). CTX is metabolized to both active DNA cross-linking species, such as phosphoramidate mustard, and to species such as acrolein which are devoid of anticancer activity (7) but which may nonetheless be toxic to normal tissues such as the urinary bladder (9, 10). Previous drug interaction studies with CTX in rodents have shown that microsomal enzyme induction with phenobarbital reduces CTX cytotoxic activity (11) whereas experimental microsomal enzyme blockade with SKF 525a increases activity (11–13). The histamine-H2 antagonist CMT, which is used widely in the treatment of peptic ulcer disease, may also interact with microsomal enzymes to impair hepatic drug metabolism (14, 15).

In a previous study of the potential interaction between CMT and CTX we showed significant CMT enhancement of CTX antileukemic activity in P388 leukemic mice (16). In addition, CMT was shown to increase CTX alkylating metabolites in these mice. This suggested that the combination of CMT and CTX in patients could lead to increased cytotoxicity from a given dose of CTX. Since CMT is also used commonly in cancer patients, the clinical combination of CMT and CTX could lead to increased CTX toxicities including myelosuppression. RNT is a more recently Food and Drug Administration approved histamine-H2 antagonist which has not demonstrated the level of microsomal enzyme inhibition seen with CMT (17, 18). This suggested that RNT might not alter the therapeutic activity or toxicity of CTX in vivo. If so, it might be combined more safely in patients requiring both drugs. Thus, the purpose of the current study was to simultaneously compare the in vivo effects of the two histamine-H2 antagonists, CMT and RNT, on the antitumor activity, pharmacokinetics, and toxicity of the model alkylating prodrg, CTX. A preliminary description of these results has been reported previously in abstract form (19).

MATERIALS AND METHODS

Mice. Male DBA/2J mice (6 to 8 weeks old; 25–30 g) were used for these studies (The Jackson Laboratory, Bar Harbor, ME). Mice were housed 5/cage, 10–15 animals per treatment group. Standard laboratory chow and slightly acidified tap water were available ad libitum. Animals in the spleen colony assay studies received sterile water with gentamicin sulfate (1 mg/ml) added to reduce enteropathic organisms.

Survival Studies. On day 0, animals were given 104 freshly harvested L1210 lymphocytic leukemia cells i.p. Drug treatments were begun 24 h after tumor implantation. Animals were observed twice daily for survival. A range of tumor inocula (103–105 cells i.p.) were evaluated in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. Supported in part by Grants CA23074 and CA17094 from the National Cancer Institute, NIH, Department of Health and Human Services, and by a grant from Glaxo, Inc., Research Triangle Park, NC. Presented in part at the 86th meeting of the American Society of Clinical Pharmacology and Therapeutics, San Antonio, TX. To whom requests for reprints should be addressed, at Arizona Cancer Center, Arizona Health Sciences Center, 1501 N. Campbell Ave., Tucson, AZ 85724.

CFU/femur = (Mean CFU/spleen) no. of cells/femur
no. of cells injected

The CFU/femur of the treated groups is divided by that of controls to account for the initial number of stem cells and to normalize for any bone marrow cell death.

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yield fractional CFU survival indices. The fractional survival data for each treatment were statistically compared using t tests for colinearity of CTX dose-response curves fit to a general least squares log-linear approximation.

Estimation of CTX Alkylating Species. Because of the complex nature of the metabolic disposition of CTX, a nonspecific colorimetric assay for alkylating species was used to quantify the production of active CTX metabolites in the plasma (24). Normal DBA/2J mice were given administration was either 5 or 30 min. At serial times up to 3 h after CTX, mice (n = 4 per time point) were sacrificed and blood was collected for separation into the plasma fraction. The NBP assay used for alkylating species was used to quantitate the production of active CTX metabolites in the plasma (24). Normal DBA/2J mice were given CTX (200 mg/kg i.p.) or without CMT or RNT according to Table 1. The interval between the histamine-H₂ antagonist and CTX administration was either 5 or 30 min. At serial times up to 3 h after CTX, mice (n = 4 per time point) were sacrificed and blood was collected for separation into the plasma fraction. The NBP assay used to quantitate active CTX species is a modification (11) of the original Freidman and Boger method which reacts alkylating species with γ-p-nitrobenzylpyridine (Sigma Chemical Co., St. Louis, MO). The acetone extractable fraction containing alkylating species forms an intense blue color with NBP which is then read spectrophotometrically at 540 nm (Perkin Elmer Lambda 3A). Within each experimental run a standard control studies showed that neither CMT nor RNT reacted with NCFU and leukemia cell CFU colonies on the surface of the spleen, adjusted for the number of nucleated bone marrow cells injected.

The effects of CTX and the histamine-H₂ antagonists on NCFU are shown in Fig. 1. There was no significant difference between the slope of the survival curves for the CTX-alone (control) and the CTX plus RNT treatment. Neither CMT nor RNT alone at the doses used in this study significantly decreased NCFU survival. In contrast, the combination of CTX plus CMT significantly increased cytotoxicity against NCFU (P < 0.05 by t tests for colinearity). This effect was more pronounced at the higher dose levels of CTX.

Pharmacokinetics of CTX Alkylating Metabolites. Initial concept was achieved approximately 15 min after injection. The subsequent elimination of alkylating activity appeared to follow a biphasic pattern with half-lives of 45 min for the early phase and a 20% increase in alkylating metabolites when it was given 5 min before CTX (P < 0.05 by SNK test) and with a 20% increase when it was given 30 min before CTX (not significant by SNK test). A statistical analysis of the postdistributive terminal elim-

RESULTS

Survival Studies. Tables 3 and 4 summarize the results of the acute and chronic histamine-H₂ antagonist-CTX survival studies, respectively. Control animals survived, on the average, 11–13 days after 10⁵ L1210 leukemia cells i.p., and CTX treatment produced significant dose dependent increments in survival (Table 3). Median survival increased to 26 days with the 100-mg/kg CTX dose (10% long term survivors) and to 32 days with the 200-mg/kg CTX dose (30% long term survivors of >2 months). The addition of either CMT or RNT to the 100 mg/kg CTX treatment did not alter overall survival. This result was obtained with both acute (Table 3) and chronic (Table 4) histamine-H₂ antagonist dosing schedules. However, with the higher CTX dose of 200 mg/kg, the addition of CMT but not RNT significantly enhanced survival of tumor bearing animals (Table 3). Both median survival (45 days) and the proportion of long term survivors (50%) were increased as a result of the acute CMT treatment (P < 0.001 by logrank analysis).

Spleen Colony Assay Studies. In untreated control animals there was a proportional log-linear increase in the number of NCFU and leukemia cell CFU colonies on the surface of the spleen, adjusted for the number of nucleated bone marrow cells injected.

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Pharmacokinetics of CTX Alkylating Metabolites. Initial control studies showed that neither CMT nor RNT reacted with the NBP reagent used to quantitate CTX alkylating metabolites. The administration of 200 mg CTX i.p. produced a peak plasma level of alkylating metabolites of about 500 A₅₄₀ units which was achieved approximately 15 min after injection. The subsequent elimination of alkylating activity appeared to follow a biphasic pattern with half-lives of 45 min for the early phase and 3.3 ± 2.2 (SD) h for the terminal elimination phase.

Fig. 2 demonstrates the time dependency of the interaction between CMT and CTX when administration was separated by 5 or 30 min, respectively. When CTX and CMT administration were separated by 30 min there was no change in the pharmacokinetic disposition of CTX alkylating metabolites in plasma. Fig. 3 shows the integrated A₅₄₀ × time areas for the different regimens. Cimetidine administration was associated with a 33% increase in alkylating metabolites when it was given 5 min before CTX (P < 0.05 by SNK test) and with a 20% increase when it was given 30 min before CTX (not significant by SNK test). A statistical analysis of the postdistributive terminal elimi-
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Fig. 1. Semilogarithmic plot of fractional NCFU survival (ordinate) versus CTX dose (abscissa). Each point represents the mean of 10 spleen colony counts (bars, SD). The lines were fit by least squares linear regression. Statistical analysis by t test showed the slope of the CTX plus CMT group (Δ) to be significantly different from the CTX control (●) or CTX plus RNT group (○). The latter two slopes were not significantly different.

In summary, these results show that the administration of CMT 5 min before CTX results in increases of both the CTX AUC and the terminal elimination half-life of alkylating activity in the plasma. The other CMT treatments (administered 30 min and 2 h before CTX), as well as all of the RNT treatments, did not significantly alter the pharmacokinetics of CTX alkylating activity (Fig. 2). The slightly elevated levels of alkylating metabolites with the 30-min CMT regimen were not significantly different from controls in terms of terminal half-life or total alkylating AUC.

DISCUSSION

In our in vivo model, ranitidine was found to have no effect on CTX antitumor activity, toxicity, or pharmacokinetics in mice. The chemical structure of ranitidine contains an aminealkylpyridine ring rather than the imidazole ring of CMT. This may explain the histamine-H2 antagonist potency differences as well as the minimal binding of RNT to a number of non-histamine receptor binding sites. For example CMT is known to bind to androgen receptors (25), to proteins of the hepatic microsomal mixed function oxidase system (26, 27), and to peripheral blood lymphocytes (28). In contrast, RNT has been found to interact minimally with these nonspecific binding sites. However, both RNT and especially CMT are reported to lower hepatic blood flow upon acute drug ingestion (29–31). A more recent study has shown that neither agent reduces hepatic blood flow when given chronically (32). This analysis suggests that

Fig. 2. Semilogarithmic plot of CTX alkylating activity (relative absorbance at 540 nm) on the ordinate versus the time in h after i.p. injection of CTX (200 mg/kg). Each point represents the mean of 4 determinations. The terminal disposition half-life of CTX A400 activity was 3.3 ± 2.2 h for the controls (●), 2.45 ± 1.15 h for CTX plus CMT (5-min interval) (Δ), and 3.3 ± 2.7 h for CTX plus RNT (5-min interval) (○). In B the A400 activity is plotted for CTX plus CMT, 30-min interval (9.1 ± 2.6 h half-life, Δ), CTX plus RNT, 30-min interval (4.0 ± 1.8 h half-life, □), and CTX-only control (○).

Fig. 3. Integrated areas CTX alkylating metabolites × time curves (AUC), for seven different regimens: CTX alone, CTX plus CMT at three different times; and CTX plus RNT at three different times. Each column represents the mean of four determinations. CTX alkylating metabolites were quantitated as NBP-reactive species with UV absorbance at 540 nm (A400). The AUCs were calculated by the trapezoidal rule.

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while acutely these drugs do reduce the hepatic extraction of indocyanine green dye, hepatic blood flow remains constant.

CTX is an anticancer agent which absolutely requires metabolic activation for both antitumor activity and for ultimate detoxification and elimination (1, 8). In previous studies, microsomal enzyme inhibitors such as SKF 525A (2-diethylaminoethyl-22-diphenylvalerate) have increased the alkylating activity and toxicity of CTX (11–13). Conversely, the induction of microsomal enzymes with phenobarbital has led to reduced CTX antitumor activity and toxicity toward normal and leukemic bone marrow stem cells (11). In a previous study of CTX activity against mice bearing P388 lymphocytic leukemia, CMT increased both CTX antitumor effects and total alkylating metabolites (16). The effect of CMT or RNT on CTX-induced bone marrow suppression was not investigated in these previous studies.

In the current study, CMT significantly augmented CTX antitumor activity against L1210-bearing mice. Unlike the previous study, this augmentation became statistically significant only at the highest CTX dose used (200 mg/kg i.p.). This result may be related to the lower overall chemosensitivity of the L1210 cell line when compared to the P388 leukemia cell line (33).

Perhaps the most significant new finding of the current work is the observation of significantly enhanced bone marrow suppression in mice given CTX plus CMT. CMT increased CTX toxicity towards NCFU in a dose dependent fashion. This suggests that the clinical addition of CMT to CTX regimens may result in greater myelosuppressive toxicity. This drug interaction pattern has not been described clinically as of yet; however, it has been observed when CMT was administered to patients receiving the alkylating nitrosourea, carmustine (34, 35). Carmustine, like CTX, is also detoxified by microsomal enzyme systems (6).

The CMT mediated increases in CTX effects on NCFU were proportional to the observed changes in the pharmacokinetics of CTX alkylating metabolites. There was also a direct temporal relationship between CMT administration and the increase in metabolite AUCs as well as the delay in metabolite elimination from the plasma. This time dependency for the interaction may be expected since the half-lives of both CMT and RNT in vivo are short [1.5–2.0 h, respectively (36, 37)].

The histamine-H2 antagonist doses used in the study (Table 2) were derived from the body surface area method of Freireich et al. (21). The calculations were based on human clinical doses and represented 52% of the maximal recommended CMT dose and 78% of the maximal recommended RNT dose. This suggests that the noninteractions seen with RNT were not due to inadequate dosing whereas the use of maximal CMT dose equivalents might have elicited an even greater augmentation of CTX biological effects.

Other anticancer agents which are highly metabolized by microsomal enzymes include the alkylating agents dacarbazine (2), hexamethylmelamine (5), and procarbazine (3, 4); the anthracycline antibiotics doxorubicin and daunorubicin (7); and the nitrosoureas carmustine and lomustine (6). It is possible that the antitumor activity and toxicity of each of these drugs could be altered by CMT. This interaction has been described experimentally in rats treated with either carmustine or lomustine plus CMT (6) and also recently in rabbits treated with doxorubicin and CMT (38). In the latter preliminary report, CMT increased the doxorubicin plasma AUC 3.8-fold and also increased the terminal doxorubicin half-life 2.5-fold. Thus, CMT appears to consistently increase the systemic availability of microsomally metabolized drugs. The recent clinical studies of chronic histamine-H2 antagonist effects on indocyanine green dye (32) clearly suggest that inhibition of CTX metabolism is due to a direct interaction of CMT with P-450 enzymes and not to reductions in hepatic blood flow.

Regardless of the specific mechanism involved, our results demonstrate that the in vivo combination of CTX with CMT, but not RNT, will result in increased alkylating activity and resultant cytotoxicity to normal and neoplastic cells. In our studies, clinically achievable histamine-H2 antagonist doses were used, and thus the results should be relevant to usual therapeutic applications of these drugs in patients. This suggests that CMT should be cautiously used, if at all, in patients receiving CTX. In instances in which a histamine-H2 antagonist is clinically indicated, RNT may comprise a safer drug to use with highly metabolized anticancer agents such as CTX. Further human clinical pharmacokinetic trials to document the apparent noninteraction of RNT and CTX are currently under way.

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