Interaction of Cimetidine but not Ranitidine with Cyclophosphamide in Mice1

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ABSTRACT

A series of experiments in DBA/2J mice evaluated the biological and pharmacokinetic interactions of the alkyllating 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 or any other antileukemic drug in which increased survival might be expected.

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 active DNA cross-linking species, such as phosphoramidemustard, 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 chronic (14-day) histamine-H2 blocker schedule was used. For the estimation of NCFU, irradiated host DBA mice were given 2, 5, or 7 x 10⁶ nucleated cells i.v. The final spleen colony data were calculated according to

\[ \text{CFU/femur} = \left( \frac{\text{Mean CFU/spleen}}{\text{no. of cells/femur}} \right) \cdot \frac{\text{no. of cells injected}}{\text{no. of cells injected}} \]

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 enteropathogenic organisms.

Survival Studies. On day 0, animals were given 10⁶ 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 (10⁵–10⁶ cells i.p.) were estimated to calibrate survival in terms of reduction in tumor body burden. All drug injections were given i.p. at 0.1 ml/10 g body weight. Survival statistics were calculated using the log rank method which accentuates late survival differences between different treatments (20).

Drugs and Doses. Cyclophosphamide (Neoars; Adria Laboratories) was diluted with sterile water for injection USP to concentrations of 5.0, 10.0, and 20 mg/ml immediately prior to use. Cimetidine was supplied as a 300-mg/2-ml ampul which was further diluted to 10 mg/ml. Table 1 outlines the drug doses and administration schedules for the three types of mouse studies performed. The time interval between the histamine-H2 antagonist and CTX administration was 5, 30, or 60 min. Thus CTX always followed the histamine-H2 antagonist even when a chronic (14-day) histamine-H2 blocker schedule was used. For this latter schedule, the histamine-H2 antagonists were given once daily beginning 4 days before tumor implantation (day 0) and ending on day 10. Table 2 describes the conversions used to adjust the murine histamine-H2 antagonist doses to clinically comparable dose levels for each agent based on the body surface area method of Freireich et al. (21).

Spleen Colony Forming Assays. To assess CTX effects on bone marrow stem cells, the murine spleen colony assays of Till and McCulloch were used (11, 16, 22, 23). For the estimation of NCFU, irradiated host DBA mice were given 2, 5, or 7 x 10⁶ nucleated cells i.v. These cells were donated from normal mice which had received CTX (50, 100, or 200 mg/kg) 24 h prior to sacrifice. The host animals were irradiated to 750 rads (whole body) using a Linac 4-mEV linear accelerator 24 h before receiving i.v. suspensions of bone marrow. The final spleen colony data were calculated according to

CFU/femur = \left( \frac{\text{Mean CFU/spleen}}{\text{no. of cells/femur}} \right) \cdot \frac{\text{no. of cells injected}}{\text{no. of cells injected}}

The CFU/femur of the treated groups is divided by that of controls to

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2The abbreviations used are: CTX, cyclophosphamide; AUC, area under the curve; CMT, cimetidine; NBP, γ-β-nitrobenzylpyridine; NCFU, normal colony forming unit (spleen); RNT, ranitidine; SKF, Smith Kline & French (Laboratories); SNK, Student-Newman-Keuls test; CFU, colony forming units.

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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 non-specific 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 CTX (200 mg/kg i.p.) with 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 color with NBP which is then read spectrophotometrically at 540 nm. The administration of 200 mg CTX i.p. produced a peak plasma concentration-time curve (AUC alkylating species areas) calculated by the NBP reagent used to quantitate CTX alkylating metabolites. 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 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 AS40 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 600 min for the late phase (Table 3). Median survival increased to 26 days with 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.

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 10^7 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).

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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 AS40 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 elimin-
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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 (Θ) or CTX plus RNT group (Ο). The latter two slopes were not significantly different.

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 A₄₅₀ activity was 3.3 ± 2.2 h for the controls (Θ), 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) (Δ, Δ). In B the A₄₅₀ 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, Δ), and CTX-only control (Θ).

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 (A₄₅₀). The AUCs were calculated by the trapezoidal rule.

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 amidinoalkylfuran 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 nonhistamine 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...
while acutely these drugs do reduce the hepatic extraction of indocyanine green dye, hepatic blood flow remains constant. Cyclophosphamide 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-diethyldiaminoethyl-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 and indocyanine green dye, hepatic blood flow remains constant. 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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REFERENCES

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