Cancer Chemother Pharmacol (1986) 18: 219–222

Cancer Chemotherapy and Pharmacology

The effects of cimetidine upon the plasma pharmacokinetics of doxorubicin in rabbits*

Dean E. Brenner[1, 2], Jerry C. Collins[1], and Kenneth R. Hande[1]

[1] Department of Medicine, The Nashville Veterans Administration Medical Center, and Vanderbilt University, Nashville, TN 37203, USA

[2] Department of Clinical Pharmacology and Experimental Therapeutics, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, NY 14263, USA

Summary. Cimetidine is an H_2 antagonist which inhibits cytochrome P-450 and reduces hepatic blood flow. To determine whether cimetidine interferes with the plasma pharmacokinetics of doxorubicin, we gave six female New Zealand rabbits doxorubicin 3 mg/kg, followed a month later by cimetidine 120 mg/kg every 12 h over 72 h and doxorubicin 3 mg/kg. Serial plasma specimens were obtained over 72 h and assayed for doxorubicin and its metabolites by high-performance liquid chromatography and fluorescence detection.

Doxorubicin plasma pharmacokinetics were prolonged after cimetidine pretreatment [AUC 0.76 ± 0.22 vs. $2.85 \pm 1.22\ \mu M \times h$, no pretreatment vs pretreatment ($p = 0.005$), half-life = 11.7 ± 6.55 vs 28.0 ± 8.16 h ($P = 0.0002$), and clearance = 0.129 ± 0.036 vs 0.036 ± 0.011 l/min^{-1} kg^{-1} ($P = 0.0007$)]. No significant differences were found between the AUCs for doxorubicinol, 7-deoxy doxorubicinol aglycone, or two unidentified nonpolar metabolites in nonpretreatment and pretreatment studies. Cimetidine increases and prolongs the plasma exposure to doxorubicin in rabbits. Doxorubicin metabolism does not appear to be affected by cimetidine.

Introduction

Cimetidine is an H_2-antagonist widely used for the treatment of peptic ulcer disease and gastric hyperacidity. This drug has been shown in vitro to inhibit cytochrome P-450 in a noncompetitive manner [24, 25]. In vitro, cimetidine prolongs the clearance, increases the half-life, and increases the areas under the curve of a diverse group of drugs, including oral anticoagulants [30], benzodiazepines [10, 21, 29], theophylline [28], anticonvulsants [1], ketoconazole [32], lidocaine [15], and beta-blocking agents [20, 27].

Experience with cimetidine-antineoplastic agent interaction is limited. Hande et al. [18] have demonstrated that cimetidine inhibits the excretion of procarbazine and hexamethylamine in rats. Recently, Harvey et al. [19] have reported that prolonged dosing (4 weeks) with cimetidine causes prolongation of 5-fluorouracil excretion and exposure (area under the curve) in humans.

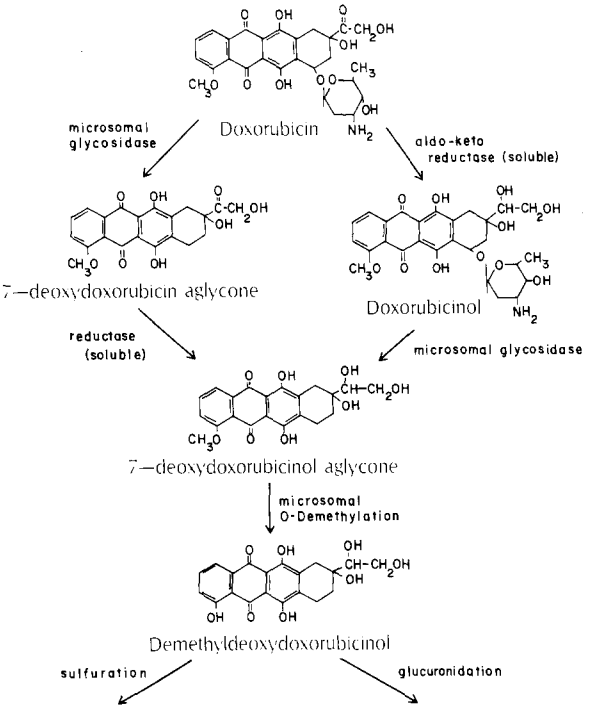

Fig. 1. Major proposed metabolic pathway of doxorubicin in humans. (Reprinted by permission, from Pratt WB and Ruddon RW, *The Anticancer Drugs, Oxford University Press, New York, 1979, p 162*

Doxorubicin (Adriamycin) is the prime example of a cytotoxic drug that is almost completely metabolized and excreted by the liver [3, 31] (Fig. 1). We put forward the hypothesis that drugs which inhibit cytochrome P-450 function or decrease hepatic blood flow would change the pharmacokinetics of hepatically handled antineoplastic agents, resulting in higher concentrations, prolonged half-lives, and increased total drug exposures similar to those changes previously observed in noncytotoxic drugs. To test this hypothesis, we have evaluated the pharmacokinetic interaction of cimetidine with doxorubicin in rabbits. Our data suggest that cimetidine increases plasma concentrations and prolongs the half-life of doxorubicin.

* Grant Support: Veterans Administration, NIH Grant RR-05424 and Clinical Research Center Grant RR-00095: American Cancer Society Institutional Grants #IN25V and IN24V, and JFCF #649

Offprint requests to: Dean E. Brenner, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY 14263, USA

Materials and methods

Materials. Doxorubicin hydrochloride was purchased as commercial material from Adria Laboratories. Tetrahydrofuran (THF) was obtained from Fisher Laboratories (HPLC grade). Ammonium formate (Fisher certified) buffer, 0.1% (w/v) was made fresh daily with distilled water and was adjusted to pH 4.0 with formic acid (Fisher certified). The buffer was subsequently filtered (Millipore, Milford, Mass) and degassed before use. For chemical extraction, chloroform (Fisher certified), 2-propanol (Fisher certified), and ammonium sulfate (Fisher certified) were used.

Synthesis of metabolites. The following metabolites were synthesized according to the published procedures of Takanashi and Bachur [31]: doxorubicinol, doxorubicin aglycone, doxorubicinol aglycone, 7-deoxydoxorubicin aglycone, and 7-deoxydoxorubicinol aglycone. Identity of the standard was confirmed by mass spectroscopy.[1] Purity of all standards was confirmed by a single peak on HPLC at published retention times [2, 8]. The lack of other peaks in the HPLC trace suggested at least 99% purity of the metabolite.

Experimental procedure. Six New Zealand white rabbits were given doxorubicin 3 mg/kg through the marginal ear vein over a 5-min period. Blood samples (3 ml) were then collected in heparinized tubes at 0.083 (5 min), 0.25, 1, 2, 4, 8, 12, 24, 36, 48, and 72 h, and immediately centrifuged at 4 °C. The plasma supernatant was removed and frozen at −20 °C until assay. All samples were assayed within 3 weeks of their collection.

Four weeks following the initial doxorubicin dose, the same rabbits received a second dose of doxorubicin, 3 mg/kg. This time, they also received cimetidine 120 mg/kg i. v. 1 h before doxorubicin and every 12 h for 72 h after the administration of doxorubicin. Blood specimens were drawn and processed as described above.

Doxorubicin assay. Specimens were assayed according to a recently described procedure [8]. Following the addition of 0.05 nmol daunorubicin per ml plasma as an internal standard, 1 ml plasma was extracted with 2 ml chloroform: isopropanol (1:1, v/v) according to the procedure of Benjamin et al. [5]. The extract was dried and stored overnight at −20 °C and reconstituted in 100 µl of a 3:7 THF:methanol solution. Then 60 ml of the plasma extract was injected into a Waters Associates (Millipore Corp., Milford Mass) dual pump, programmed-gradient high-performance liquid chromatograph. Doxorubicin and metabolites were separated on a 30 cm, 10 µm particle size, µ-Bondapak phenyl column (Waters Associates, Millipore Corp., Milford Mass) using a mobile phase consisting of THF:16 m*M*, pH 4.0 formate buffer (1:1, v/v). Metabolites were eluted over a 10-min linear gradient from 15% THF to 50% THF, with a 5-min washout period at 50% THF. Doxorubicin and metabolite peaks were detected on a Perkin-Elmer model 650-40 spectrofluorimeter fitted with an 18-µl flow cell. The excitation wavelength was 476 nm and the emission detection wavelength was 550 nm.

[1] Performed on a Nermag R10-10-R quadrapole mass spectrometer by Dr Ian Blair

Peaks were printed and integrated on a Waters Associates (Millipore Corp., Milford, Mass) data module. Doxorubicin and metabolites were identified according to peak retention times compared with standards injected in methanol. The assay is sensitive to 0.054 ng doxorubicin injected from a methanol solution and 2 ng doxorubicin extracted from human plasma [8].

Quantitation. A standard curve was prepared from each rabbit's pretreatment plasma. The ratio of known doxorubicin peak areas to a 0.05 nmol/ml daunorubicin internal standard was calculated. Following linear regression analysis, concentrations of metabolites and doxorubicin from plasma were calculated from their ratio to the internal standard.

Pharmacokinetics. Doxorubicin concentrations were fitted by means of the nonlinear curve fitting program MLAB [21] to the equation:

$$C_{(t)} = Ae^{-\alpha t} + Be^{-\beta t},$$

where $C_{(t)}$ is the plasma concentration at time t, A and B are constants, and α and β are linear elimination rate constants. A $1/(\text{concentration})^2$ weighting function was used for curve fitting. The area under the curve (AUC) was calculated for doxorubicin by the log trapezoidal rule to infinity. Clearance was calculated by:

$$D_o/AUC,$$

where D_o is the administered dose. Volume of distribution was calculated by a noncompartmental method [4]:

$$V_{dss} = D_o\,(AUMC)/AUC^2$$

where AUMC is the area under the movement curve. Areas under the curve for metabolites were calculated by the log-trapezoidal rule. Student's *t*-test for paired means was used to test for statistical significance.

Results

Two-compartment fits of doxorubicin concentrations with and without cimetidine are shown in Fig. 2. Pharmacokinetic data are listed in Table 1. Significant differences were found in all major pharmacokinetic parameters. Cimetidine prolonged the half-life and increased the area under the curve. The calculated total-body clearance was reduced.

Doxorubicinol, a combination peak of 7-deoxydoxorubicinol aglycone and doxorubicin aglycone, and unknown metabolites C and G were detected in most rabbits (Table 2). There were no differences from baseline values in metabolite exposure when the animals were treated with cimetidine. The wide standard deviations seen in metabolite AUCs reflect their low concentrations at or near the detection limits of the assay (0.005 µ*M* doxorubicin fluorescent equivalent). Plasma concentrations of the metabolites were neither as high nor as long in duration as we have found in human plasma [6].

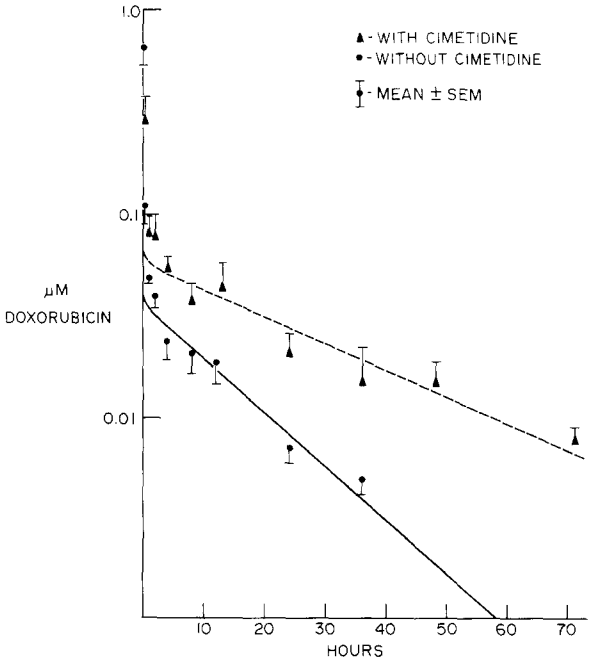

Fig. 2. Concentration time curve of doxorubicin plasma concentrations obtained from New Zealand White rabbits pretreated with cimetidine 1 h before and every 12 h for 72 h after doxorubicin administration, compared with the concentrations obtained from the same rabbits without cimetidine treatment

Discussion

In view of the widespread use of doxorubicin as anticancer therapy, it is important to consider the possibility of drug-drug interactions with commonly used drugs such as cimetidine. Cimetidine's interactions with multiple drugs are well described. At least four mechanisms have been identified. First, cimetidine is thought to affect gastrointestinal-absorption of ketoconazole and penicillin owing to its H_2 receptor antagonism and subsequent decrease in gastric acid secretion [13, 32]. Second, cimetimetidine-induced H_2 receptor blockade decreases hepatic blood flow. Reduced hepatic uptake of propanolol due to decreased blood flow has resulted in increased plasma drug concentration and area under the curve [15, 20, 27]. Third, the inhibition of cytochrome P-450 by cimetdine is described as noncompetitive [24] and has been documented in vitro [24] and in vivo with multiple pharmacologic agents [1, 10, 21, 28–30]. Finally, preliminary data have suggested that cimetidine causes competitive inhibition of renal tubular secretion of organic acids [23], theoretically interacting with drugs secreted by the kidney. No such interactions have been reported to date.

Drug interactions with doxorubicin are well documented. Reich and Bachur [26] initially described phenobarbital-enhanced metabolism and clearance of doxorubicin in mice. Subsequent human studies by Chang et al. [9] suggested that streptozotocin delayed doxorubicin excretion and caused increased myelotoxicity. Streptozotocin-induced hepatotoxicity was thought to cause delay in the metabolism of doxorubicin and hence delayed drug excretion and toxicity. Studies conducted by Evans et al. [12] in children have found that cyclophosphamide prolongs the plasma exposure of doxorubicin. The increased doxorubicin exposure is probably due to 4-hydroxycyclophosphamide, a major metabolite of cyclophosphamide, which may have induced inhibition of cytochrome P-450 reductase activity [11]. This drug interaction has been shown to delay the appearance of 7-deoxydoxorubicinol aglycone and 7-deoxydoxorubicin aglycone [11].

Doxorubicin disappears from plasma faster in rabbits than in humans. Rabbits metabolize the drug to aglycones in a manner similar to man, albeit in low concentrations [7]. Our study shows that cimetidine induces a significant decrease in doxorubicin clearance in rabbits. This is also reflected in an increased terminal excretion half-life and an increased area under the curve. Since doxorubicin is not administered orally or excreted in large quantities by the kidney, these two mechanisms of drug-drug interaction are not relevant. Hepatic extraction efficiency of doxorubicin in humans is approximately 50%–60% [16]; the extraction efficiency in rabbits, to our knowledge, is un-

Table 1. Pharmacokinetic parameters obtained from biexponential analysis of doxorubicin plasma-time profile

	AUC (μ*M*-h)	$T_{1/2}$ (h)	Cl_{TB} (l/min^{-1} kg^{-1})	V_{dss} (l/kg)
Doxorubicin ($n = 6$)	0.76 ± 0.22	11.7 ± 6.55	0.129 ± 0.036	112.4 ± 77.0
Doxorubicin + cimetidine ($n = 6$)	2.85 ± 1.22	28.0 ± 8.16	0.036 ± 0.011	80.5 ± 38.8
$P =$	0.005	0.002	0.0007	0.23

Values given are means ± SD

Table 2. Areas under the concentration-time curve for doxorubicin and metabolites in animals receiving doxorubicin with or without cimetidine

Metabolite	Doxorubicin alone (μ*M*-h)	Doxorubicin + cimetidine (μ*M*-h)	*P*
Doxorubicin	0.750 ± 0.22	2.850 ± 1.22	0.005
Doxorubicinol	0.450 ± 0.28	0.590 ± 0.22	0.32
7-Deoxydoxorubicinol aglycone	1.010 ± 1.28	1.460 ± 3.34	0.71
Unknown metabolite C	0.440 ± 0.036	0.065 ± 0.065	0.58
Unknown metabolite G	0.026 ± 0.015	0.060 ± 0.051	0.14

Values given are means ± SD

known. Since hepatic blood flow changes induced by histamine blockade affect only high-clearance drugs [15, 20, 27] (such as propranolol), it is not likely that this mechanism is primarily responsible for the large clearance delay we have observed.

This leaves us with inhibition of cytochrome P-450 as the primary mechanism of cimetidine's delay of doxorubicin clearance. Unfortunately, the metabolite data do not support this assertion. If cytochrome P-450 enzymes had been blocked, then higher doxorubicinol exposure and lower aglycone metabolite concentrations in the cimetidine-treated animals would have been observed. Instead, no significant differences in doxorubicinol or aglycone exposures between the two treatments was found. These discrepancies may be explained by a trend toward higher doxorubicinol concentrations and by the low metabolite concentrations which were, with the exception of doxorubicinol, at the detection limits of our assay. The large standard deviations of areas under the curve support this assertion [14]. Finally, it is possible that cimetidine inhibits the aldehyde and ketone reductases, which convert doxorubicin to doxorubicinol.

Further work evaluating the interactions of cimetidine with antineoplastic agents is important. The widespread use of this drug and its ability to inhibit cytochrome P-450 enzymes may have adverse clinical effects upon patients receiving other hepatically metabolized antineoplastic agents.

Acknowledgements. We wish to acknowledge the assistance of Mr Brendan McAllister with the log trapezoidal programs, of Dr Ian Blair with mass spectrographic analyses, of Dr Lowell Anthony for assistance with the study, of Ms Rita Bennett and Louis Combs for technical assistance,and of Ms Terry Hara.

References

1. Algozzine GJ, Stewart RB, Springer PK (1981) Decreased clearance of phenytoin with cimetidine. Ann Intern Med 95: 244–245
2. Andrews PA, Brenner DE, Chou FTE, Kubo H, Bachur NR (1980) Facile and definitive determination of human adriamycin and daunorubicin metabolites by high-pressure liquid chromatography. Drug Metab Dispos 8: 152–156
3. Bachur NR (1979) Anthracycline antibiotic pharmacology and metabolish. Cancer Treat Rep 63: 817–820
4. Benet LZ, Babeazzi RL (1979) Noncompartmental determination of the steady-state volume of distribution. J Pharm Sci 68: 1071–1074
5. Benjamin RS, Riggs CE, Bachur NR (1977) Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function. Cancer Res 37: 1416–1420
6. Brenner DE, Grosh WW, Noone R, Stein R, Greco FA, Hande KR (1984a) Human plasma pharmacokinetics of doxorubicin: Comparison of bolus and infusional administration. Cancer Treat Symp 3: 77–83
7. Brenner DE, Noone R, Hande KR (1984b) A comparison of doxorubicin pharmacokinetics in the rabbit and in man. Proc Am Assoc Cancer Res 28: 300
8. Brenner DE, Galloway S, Cooper J, Noone R, Hande KR (1985) Improved high-performance liquid chromatography assay of doxorubicin: Detection of circulating aglycones in human plasma and comparison with thin-layer chromatography. Cancer Chemother Pharmacol 14: 139–145
9. Chang P, Riggs CE, Scheerer BA, Wiernik PH, Bachur NR (1976) Combination chemotherapy with adriamycin and streptozotocin. Clin Pharmacol Ther 20: 611–616
10. Desmond PV, Patwardhan RN, Schenker S, Speeg KV (1980) Cimetidine impairs the elimination of chlordiazepoxide (Librium) in man. Ann Intern Med 93: 266–268
11. Dodion P, Riggs CE, Akman SR, Tamburini JM, Colvin OM, Bachur NR (1984) Interactions between cyclophosphamide and adriamycin metabolism in rats. J Pharmacol Exp Ther 229: 51–57
12. Evans WE, Crom WR, Yee G, Green AA, Hayes FA, Pratt CB, Avery TL (1980) Adriamycin pharmacokinetics in children. Proc ASCO AACR 21: 176
13. Fairfax AJ, Adam J, Pagan FS (1978) Effect of cimetidine on the absorption of oral benzylpenicillin. Br Med J 1: 820
14. Felsted RL, Bachur NR (1980) Mammalian carbonyl reductase. Drug Metab Rev 11: 1–60
15. Freely J, Wilkinson GR, McAllister CB, Wood AJJ (1980) Increased toxicity and reduced clearance of lidocaine by cimetidine. Ann Intern Med 96: 592–594
16. Garnick MB, Ensminger WD, Israel M (1979) A clinical pharmacological evaluation of hepatic arterial infusion of adriamycin. Cancer Res 39: 4105–4110
17. Greene RF, Collins JM, Jenkins JF, Speyer JL, Myers CE (1983) Plasma pharmacokinetics of adriamycin and adriamycinol: Implication for the design of in vitro experiments and treatment protocols. Cancer Res 43: 3417–3421
18. Hande KR, Noone RM (1983) Cimetidine prolongs the half-life of procarbazine and hexamethylmelamine. Proc Am Assoc Cancer Res 24: 287
19. Harvey VJ, Slevin ML, Dilloway MR, et al (1984) The influence of cimetidine on the pharmacokientics of 5-fluorouracil. Br J Clin Pharmacol 18: 421–430
20. Kirch W, Kohler H, Spahn H, Mutschler E (1981) Interaction of cimetidine with metoprolol, propranolol, or atenolol. Lancet 2: 531–532
21. Klotz U, Reimann I (1980) Influence of cimetidine on the pharmacokinetics of desmethyldiazepam and oxazepam. Eur J Clin Pharmacol 18: 517–520
22. Knott GD (1979) MLAB – A mathematical modeling tool. Comput Programs Biomed 10: 271–280
23. McKinney TD Myers P, Speeg KV Jr (1981) Renal organic base transport Cimetidine secretion by proximal straight tubules. Clin Res 29: 471A
24. Pelkonen O, Puurunen J (1980) The effect of cimetidine on in vitro and in vivo microsomal drug metabolism in the rat. Biochem Pharm 29: 3075–3080
25. Puurunen J, Sotaniemi E, Pelkonen O (1980) Effect of cimetidine on microsomal drug metabolism in man. Eur J Clin Pharmacol 18: 185–187
26. Reich SD, Bachur NR (1976) Alterations in adriamycin efficacy by phenobarbital. Cancer Res 36: 3803–3806
27. Reimann IW, Klotz U, Siems B, Frolich JC (1981) Cimetidine increases steady state plasma levels of propranolol. Br J Clin Pharmacol 12: 785–790
28. Roberts RK, Grice J, Wood L, Petroff V, McGuffie C (1981) Cimetidine impairs the elimination of theophylline and antipyrine. Gastroenterology 81: 19–21
29. Ruffalo RL, Thompson JF, Segal JL (1981) Diazepam-benzodiazepine drug interaction. Am J Hosp Pharm 38: 1365–1366
30. Silver BA, Bell WR (1979) Cimetidine protentiation of the hypoprothominemic effect of warfarin. Ann Intern Med 90: 348–349
31. Takanashi S, Bachur NR (1976) Adriamycin metabolism in man: Evidence from urinary metabolites. Drug Metab Dispos 4: 79–97
32. Van der Meer JWM, Keuning JJ, Scheijgrond HW, Heykants J, Van Cutsem J, Brugmans J (1980) The influence of gastric acidity on the bioavailability of ketoconazole. J Antimicrob Chemother 6: 552–554

Received December 3, 1985/Accepted July 21, 1986