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Pharmacokinetics of Carminomycin in Dogs and Humans

Preliminary Report

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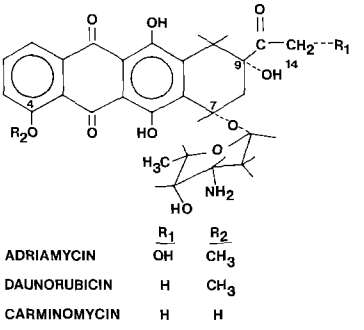
Summary. *Carminomycin was administered to four dogs and two human patients as a single intravenous dose. Plasma samples were obtained and assayed for carminomycin and carminomycinol by high pressure liquid chromatography with fluorescence detection. The plasma disappearance of carminomycin could be described by a three-compartment open model. Distribution was rapid and the apparent volume of distribution was greater than 100 l/m² in both species. The terminal half-life of drug was 86 h in dogs and 20 h in humans. In both dogs and humans carminomycinol concentrations rapidly surpassed carminomycin levels, and terminal half-lives were longer than for the parent compound in the two species. Since carminomycinol has antitumor activity and host toxicity, this metabolite may play an important role in the efficacy and toxicity of carminomycin therapy.*

Introduction

Carminomycin (carubicin, NSC 180024) is an anthracycline antibiotic related to adriamycin (doxorubicin, NSC 123127) and daunorubicin (NSC 82151). These agents differ in chemical structure at carbons 4 and 14, as shown in Fig. 1. Clinical studies of carminomycin in the Soviet Union have demonstrated activity against a variety of solid tumors, including breast carcinoma and soft-tissue sarcomas, as well as activity against hematologic neoplasms [10, 11]. Phase I investigations have been undertaken in the United States [5, 8] and Belgium [1] as a first step in verifying the clinical spectrum of activity and toxicity as found by the studies performed in the Soviet Union.



	R_1	R_2
ADRIAMYCIN	OH	CH_3
DAUNORUBICIN	H	CH_3
CARMINOMYCIN	H	H

Fig. 1. Chemical structure of adriamycin, daunorubicin, and carminomycin

Single intravenous (IV) doses of carminomycin were administerd to patients with solid neoplasms at SUNY-Upstate Medical Center, as reported by Comis et al. [8]. Blood samples were obtained from all patients during their initial course of therapy. Unfortunately, due to a long storage period (> 6 months) between collection of samples and assay, samples deteriorated even though frozen at −10° C. Two patients were restudied after six courses of therapy at the dose level of 15 mg/m² and the samples were analyzed promptly. The study was temporarily halted by the Food and Drug Administration for technical reasons, so no other patients were available.

In an attempt to further delineate the pharmacokinetics of carminomycin, the drug was administered to dogs as a single IV injection. The results of the pharmacokinetic evaluation of two patients and four dogs given IV single-dose carminomycin are the basis of this preliminary report.

Methods and Materials

Patients. The two patients selected for study had histologically proven, disseminated, solid, malignant tumors, and had received

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multiple courses of carminomycin at a dose level of 15 mg/m^2. One patient had a renal cell carcinoma with metastases to the lung and the other had malignant melanoma. Both patients had given informed consent before receiving the drug. At the time of the study these patients had had stable disease for 7.5 and 8 months, respectively, while receiving carminomycin. Both patients were ambulatory and had no clinical or laboratory evidence of liver or renal dysfunction. The previous dose of carminomycin had been given 4 weeks before.

Dogs. Male and female beagle dogs weighing 9–14 kg were obtained from Marshall Farms (North Rose, NY). These dogs were in good health according to an examination of their hematological and blood chemistry tests. Body surface area was estimated from body weight, and drug administered at a dose level of 7.5 mg free base/m^2, which is equivalent to about 8.0 mg carminomycin hydrochloride/m^2.

Drug. Carminomycin hydrochloride was obtained from Russian sources and determined by high pressure liquid chromatography to be at least 95% pure. For these studies, the drug was formulated with 200 mg mannitol and 10.45 mg carminomycin hydrochloride (10 mg carminomycin base) per vial. Solutions were prepared immediately prior to dosing. Carminomycinol was obtained by borohydride reduction of carminomycin according to the procedure of Povarov et al. [12].

Assay. The assay procedure of Averbuch et al. [3] was employed, and a brief description follows. Plasma, obtained from heparinized blood, was taken from storage at −10° C and thawed. Adriamycin (400 ng) (Adria Laboratories, Dublin, OH) was added as internal standard to 1.0-ml aliquots of test plasma. To each plasma sample, two volumes of chlorform : isopropranol (1 : 1 V/V) were added to extract drug and metabolites. Saturating quantities of solid ammonium sulfate were used to salt the aqueous phase. The mixture was centrifuged (2,650 × *g* for 30 min) and the organic layer removed. Evaporation of the organic solvent was done under a nitrogen gas stream. The residue was reconstituted in 50 µl chloroform : methanol : acetic acid : water (80 : 20 : 2 : 3), and injected into a high pressure liquid chromatograph (HPLC). The HPLC consisted of a U6K injector (Waters Associates, Milford, MA, USA), an Altex pump (Model 110, Rainin Instrument Co., Brighton, MA, USA), a Partisil-10PAC column (PXS 10/25 PAC, Whatman, Inc., Clifton, NJ, USA), a Spectro/Glo filter fluorometer (Gilson Medical Electronics, Middleton, WI, USA), and a linear chart recorder (Serco, Inc., Deerfield, IL, USA). The fluorometer was equipped with an emission filter of bandwidth 565–600 nm and excitation filter of bandwidth 380–480 nm. Flow cell volume was 15 µl.

The mobile phase of chloroform : methanol : acetic acid : water (80 : 20 : 2 : 3) was pumped through the column at a constant flow rate of 3.5 ml/min. At these conditions the retention times of carminomycin, carminomycinol and adriamycin were 2.3, 3.3, and 3.9 min, respectively.

The gain control of the fluorometer was adjusted during chromatographic separation to keep the adriamycin standard peak on scale. The gain was linear over the range of attenuation. Peak heights of carminomycin and the major metabolite, carminomycinol, were compared to the peak height of adriamycin without correction for retention times. All peak heights were normalized by dividing the peak height by the sensitivity setting (gain control) of the fluorescence detector. Standard curves were prepared at various concentrations of carminomycin and carminomycinol extracted from pooled human or dog plasma. Carminomycin concentrations were compared with the adriamycin (400 ng/ml) peak. However, because of the proximity of the adriamycin and carminomycinol peaks a considerable amount of peak overlap sometimes occurred. A carminomycinol standard curve was prepared, therefore, with a fixed amount of carminomycin (50 ng). The carminomycinol peaks of test samples were first normalized in terms of carminomycin equivalents by running test samples without the internal standard. A ratio of carminomycinol to carminomycin peak heights was obtained. The carminomycinol standard curve was used to allow the carminomycinol concentration to be expressed as carminomycin equivalents. The samples were run again with adriamycin as internal standard, and concentrations for both carminomycin and carminomycinol calculated.

Mathematical Modeling. The SAAM 27 computer program from the National Institutes of Health [6] was used to model the plasma disappearance curves. On the basis of visual inspection of the curves, a three-compartment open model for the dog study and a two-compartment open model for the human data were chosen as initial models. The data from the human studies were analyzed individually with a two-compartment model, and also with a three-compartment model, when the average values for the two patients were used.

Results

Standard curves for carminomycin and carminomycinol were linear over the ranges tested, which were 0–50 ng/ml for carminomycin and 0–80 ng/ml for carminomycinol. Response was expressed as a fraction of peak height of carminomycin to adriamycin internal standard or carminomycinol to carminomycin. The correlation coefficients of best fit lines by linear regression of response versus concentration were greater than 0.990 when four or five points were run in triplicate. Since these standard curves remained relatively constant from day to day, full standard curves were not run with all test samples. Instead, only three or four standards were run to check linearity prior to performing analysis of unknown plasma samples.

Absolute molar fluorescence ratios have not been obtained for carminomycin and carminomycinol as compared to adriamycin with the Gilson fluorometer. However, carminomycin had a greater fluorescence in this HPLC system than adriamycin, and carminomycinol had a decreased fluorescence compared with carminomycin. A concentration of 40 ng carminomycin/ml, which was one-tenth the concentration of the adriamycin internal standard, gave a response equivalent to 110 ng adriamycin/ml. A concentration of 50 ng carminomycinol/ml gave a response equivalent to only 13 ng carminomycin/ml.

Plasma disappearance curves of carminomycin and its chief metabolite, carminomycinol, are shown in Fig. 2 for dogs and Fig. 3 for the two patients. The points represent the average of the values obtained at each sampling time for each species. Blood samples were obtained during the 72-h period after dosing of the dogs. However, only concentrations of carmino-

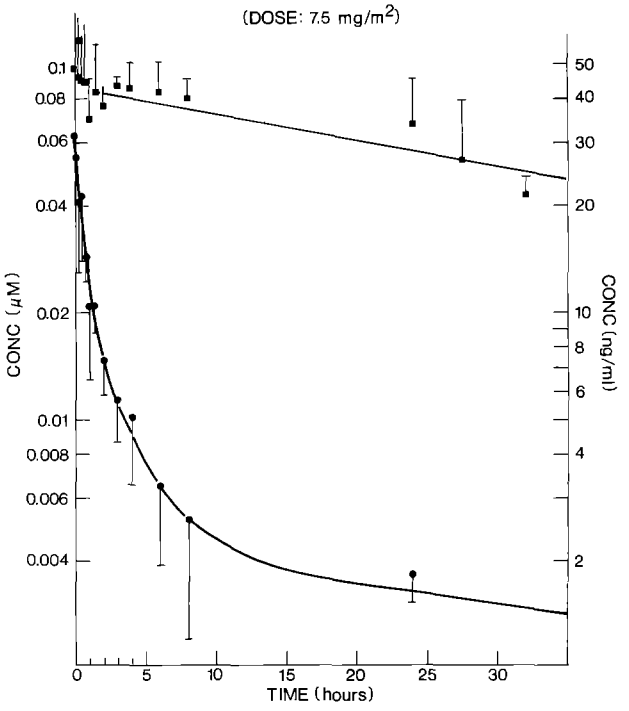

Fig. 2. Concentration versus time curves for carminomycin (●) and carminomycinol (■) in dogs. Carminomycin was administered as a single IV injection at a dose of 7.5 mg/m^2. *Bars* represent standard deviation about the mean of values from four dogs

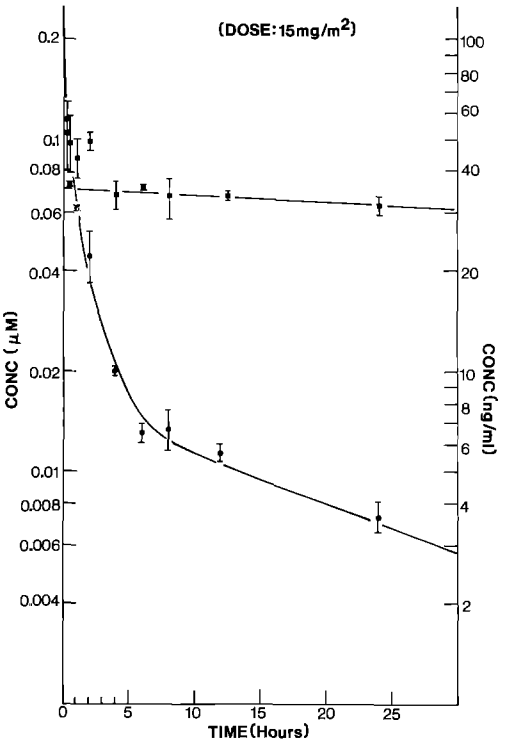

Fig. 3. Concentration versus time curves for carminomycin (●) and carminomycinol (■) in patients. Carminomycin was administered as a single IV injection at a dose of 15 mg/m^2. *Bars* represent the range of the values for the two patients studied

mycin during the first 32 h were used for pharmacokinetic modeling, since concentrations of carminomycin beyond 24 h were near the limits of sensitivity of the assay. Modeling of the plasma concentrations obtained from patients was done from all time points, but sampling was restricted to 24 h.

Carminomycinol concentrations rapidly reach peak levels and surpass carminomycin concentrations within a short period of time in the patients and dogs. Since the elimination phase of carminomycinol is comparable to that of carminomycin, the carminomycinol concentrations remain elevated compared with parent drug concentrations. The data are not precise enough to accurately describe the early kinetics of the carminomycinol concentration curve.

Carminomycin disappearance from the plasma of dogs can best be described by a triphasic curve, which can be represented by a three-compartment open model. However, a biphasic curve adequately described the kinetics of carminomycin disappearance in the two patients. When the individual plasma curves of the patients were subjected to analysis by a two-compartment model, the best fit curves had a sum-of-squares value of 7.1 and 5.5. If a three-compartment model and average concentrations were used, then the sum-of-squares value decreased to 4.3. A sum-of-squares value of 2.0 was obtained for a three-compartment model when average concentrations from the dog study were used. The results of modeling the pharmacokinetics of carminomycin with the SAAM computer program are shown in Table 1.

Table 1. Rate constants determined for carminomycin kinetics

Species	A. Three-compartment model[b]				
	K_{12}	K_{21}	K_{13}	K_{31}	K_{10}
Dog	0.837	1.002	0.543	0.0518	0.114
Human	2.312	3.298	0.728	0.120	0.35
Patient	B. Two-compartment model[b]				
	K_{12}	K_{21}	K_{10}		
1	0.369	0.11	0.182		
2	0.518	0.137	0.164		

[a] Kij = rate of transfer from compartment i to compartment j in h^{-1}

[b] Central compartment = 1; peripheral compartments = 2 and 3; elimination = 0

The plasma concentrations from dogs and patients can be described by an equation which is the

Table 2. Pharmacokinetic values for carminomycin

Parameters	Species	
	Human	Dog
P (ng/ml)	54.9	21.1
A (ng/ml)	45.1	10.3
B (ng/ml)	7.9	2.0
π (h^{-1})	6.10	2.20
α (h^{-1})	0.67	0.34
β (h^{-1})	0.034	0.008
$t_{1/2}$ π (h)	0.11	0.31
$t_{1/2}$ α (h)	1.03	0.43
$t_{1/2}$ β (h)	20.38	86.34
Vd (l/m^2)	139	225

sum of three exponential terms. This equation has the form of: $C = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$. The values of the constants for both dog and human data are given in Table 2. These values as well as the half-lives of the three phases were calculated by standard techniques on the basis of the intercompartmental rate constants determined by the computer program. The half-life of the terminal phase of the carminomycinol concentration curves was 32 h for the dogs and 6 days for the patients. Terminal half-lives were calculated by linear regression of the log of the concentrations from 4–32 h for the dogs and 4–24 h for the patients. Because of the short sampling time, this phase may not reflect the true terminal phase half-life of the drug or metabolite.

Discussion

Carminomycin is similar in molecular structure to adriamycin and daunorubicin as shown in Fig. 1. These drugs have a ketone group on carbon-13 which can be metabolized to the corresponding alcohol by an intracellular aldo-keto reductase [4]. This enzyme has been found in the cytoplasm of all mammalian cells which have been studied. Daunorubicin is a better substrate for this enzyme than adriamycin, and preliminary data (L. Martinez, personal communication) suggest that carminomycin is a better substrate than daunorubicin.

The finding of high carminomycinol concentrations in the plasma of dogs and patients receiving carminomycin is significant, since carminomycinol has known antitumor activity and host toxicity. The antitumor activity of carminomycinol isolated from a strain of *Streptomyces peucetius* was compared with that of daunorubicin, daunorubicinol, and carminomycin by Cassinelli et al. [7]. The concentrations that inhibited cloning efficiency by 50% of HeLa cells after treatment for 24 h in vitro were 0.01 and 0.02 μM for daunorubicin and daunorubicinol, and 0.006 and 0.008 μM for carminomycin and carminomycinol, respectively. The optimal dose against L-1210 leukemia in vivo was 4.4 mg/kg for both daunorubicin and daunorubicinol, while only 0.6 and 0.4 mg/kg were needed for carminomycin and carminomycinol. The (median survival time of treated mice/median survival time of control mice) × 100, or T/C%, was 162 for daunorubicin, carminomycin, and carminomycinol, but 138 for daunorubicinol. There appeared to be a slight loss of in vivo activity by conversion of the carbon-13 ketone to the alcohol in the daunorubicin series, but not in the carminomycin series.

The toxicity and antitumor activity of carminomycinol prepared chemically by borohydride reduction of carminomycin was studied by Povarov et al. [12]. Carminomycin was slightly more active than carminomycinol against a variety of transplanted tumors in mice. The toxicity to white mice given a single IV injection of drug was almost the same for carminomycin and carminomycinol. The LD_{50} (dose lethal to 50% of animals) values for carminomycin and carminomycinol were 3.5 and 3.6 mg/kg, respectively. The lethal effect of carminomycinol was slightly delayed compared with that of carminomycin.

At present it is not clear whether the high concentrations of carminomycinol are due to a high conversion rate compared with adriamycin or to markedly different distribution properties of carminomycinol compared with adriamycinol. A combination of these factors may play a role. The current working hypothesis is that carminomycin is rapidly and extensively converted to carminomycinol by an intracellular enzymatic process. Carminomycin and carminomycinol would then both be responsible for cell death.

A preliminary report [9] of the plasma concentration curves of the two patients who received carminomycin showed carminomycinol concentrations which were about 2.5 times lower than the correct values. The concentrations of carminomycinol were calculated on the assumption that there was equal molar fluorescence and similar solvent partition coefficients for carminomycin and carminomycinol. This assumption was made since adriamycin and adriamycinol as well as daunorubicin and daunorubicinol have been reported to behave similarly [2]. To confirm that the high levels of carminomycinol were not due to an artifact caused by the assay system, carminomycinol was synthesized. Direct comparison with carminomycin demonstrated the lower fluorescence of carminomycinol in the assay system.

The dog appears to be an appropriate model for the study of the clinical pharmacokinetics of carminomycin and carminomycinol, since plasma concentration curves in this species resemble those found in humans. Although a two-compartment system is adequate to model the carminomycin concentration data from the patients, a three-compartment model was employed because of the information derived from the dog study. It was assumed that the rapid, initial distribution phase, referred to as the π-phase, was missed during the human study, since plasma was not sampled at early time points as it was in the dog study. The sum-of-squares value obtained from the SAAM 27 program suggested that a three-compartment model describes the carminomycin concentration data better than a two-compartment model.

Even though rate constants, volumes of distribution, and elimination half-lives for carminomycin and carminomycinol differ somewhat between dogs and humans, it is clear that carminomycinol is a major metabolite in both species. The fact that high concentrations of carminomycinol were detected in dogs and humans and that carminomycinol is biologically active suggests that this metabolite plays an important role in the efficacy and toxicity of carminomycin. Further studies on the molecular and clinical pharmacology of this metabolite are warranted.

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