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Plasma Concentrations of Carminomycin and Carminomycinol in Man, Measured by High Pressure Liquid Chromatography*

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Abstract—*In 9 patients with advanced malignant disease who received carminomycin (CMM) in an i.v. bolus injection (dose* 18 mg/m^2*), curves of plasma concentrations of CMM and carminomycinol (CMMOH), a metabolite, versus time were constructed. For determination of plasma concentrations, high pressure liquid chromatography was used. For CMM and CMMOH the median areas under the curves (AUC's) were* 31 *(range* 4–57) × 10^{-8} mol/l/hr *(measured over* 24 hr*) and* 100 *(range* 30–158) × 10^{-8} mol/l/hr *(measured over* 48 hr*) respectively. From the data an accumulation of CMMOH in patients receiving treatments separated by brief intervals can be predicted (half-life time of plasma disappearance for CMMOH was* 2 days*). Clinical toxicity was lowest in those 3 patients showing the lowest AUC for both CMM and CMMOH.*

INTRODUCTION

CARMINOMYCIN (CMM) is one of a group of new anthracyclines. It was developed with a view to minimising cardiac toxicity while maintaining the antitumor potency of adriamycin [1]. A pharmacokinetic study was designed to find out more about the pharmacology and toxicology of the analogue CMM while it was undergoing phase II trial in patients. The first requirement for pharmacokinetics is a drug assay, and so we have used a high-pressure liquid chromatography assay described by Fandrich and Pittman [2] for the determination of CMM and carminomycinol (CMMOH), a metabolite of CMM. They showed plasma concentrations of one patient after receiving CMM. The CMMOH : CMM plasma concentration ratio was 10, measured 24 hr after injection. As CMMOH is reported to show antitumor activity in mice [3], pharmacokinetic studies are necessary in more patients.

Our assay method was modified in several ways, most importantly leading to reduction in the sample clean-up time. Plasma samples were obtained from cancer patients whose subsequent drug-related toxicity and antitumor response were closely documented, thus affording possibilities for clinical pharmacological analysis.

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MATERIALS AND METHODS

CMMOH was kindly provided by Bristol Laboratories (Syracuse, NY, U.S.A.) and 4'-epi-adriamycin (4'-epi-ADM) by Farmitalia (Milan, Italy). For injection, CMM (vials from Bristol containing 10 mg CMM + 20 mg mannitol) was dissolved in 20 ml glucose (5%). Heparinized blood (4 ml) was collected for each sample and centrifuged within 10 min. Plasma samples were stored at −20°C.

Sample clean-up and chromatographic conditions

4'-Epi-ADM was added to plasma as an internal standard for the assay (final concentration, 4.3×10^{-7} M). Plasma samples were kept in ice prior to extraction. Plasma (1 ml) was vortexed carefully with 5 ml of a mixture of isopropanol/chloroform (1 : 4, v/v). The organic layer was removed and evaporated to dryness within 20 min with air at 35°C. The plasma was extracted with another 5 ml of organic solvent. The residue was dissolved in 150 μl of methanol. The use of eluent in place of methanol resulted in a turbid solution. Of this

solution, 100 μl was injected onto the column. A C18 reverse-phase column was used (ODS, Waters Assoc., particle size 10 μm) with column dimensions: length 25 cm and i.d. 4.6 mm. The mobile phase consisted of phosphoric acid (10^{-2} M, brought to pH 4 with KOH) and acetonitrile (3:2, v/v).

The flow rate was 2 ml/min at a pressure of 6 MPa. For detection, a fluorometric monitor (model 3000, Perkin Elmer) was used (excitation and emission wavelengths were 490 nm and 550 nm). The detection limit was 5×10^{-9} M. Capacity factors for 4'-epi-ADM, CMMOH and CMM were 5.8, 8.5 and 10.9 respectively. In order to decrease adsorption, all glassware was treated with dichlorodimethylsilane (5% in chloroform). All chemicals were of analytical grade.

Stability of solutions

In water solutions of 4'-epi-ADM, CMM and CMMOH appeared to be unstable due to light and temperature influences. This has been previously described for anthracyclines [2, 4]. CMM and CMMOH were more labile than 4'-epi-ADM and declined to 28, 32 and 57% of the initial concentration (1.8×10^{-4} M) respectively in 20 hr at 25°C.

Freezing and thawing resulted in a loss of 12% for CMM and CMMOH. Reaction products, coming unretained from the column, could be detected at a wavelength of 254 nm. They were not fluorescent. In plasma the stability of these anthracyclines was not affected under the conditions of the assay and also, no loss was found after three-fold freezing and thawing.

Patients

Patients participating in this study were treated according to protocols of the European Organization for Research on the Treatment of Cancer (EORTC). One patient (B) had soft tissue sarcoma. The other patients had metastatic breast cancer. In all patients, pre-treatment Karnofsky performance status was more than 70%. Toxicity due to CMM was expressed using the WHO grades [5], which were summated per course and averaged.

RESULTS AND DISCUSSION

Plasma concentrations of CMM in 9 patients who received the drug in an i.v. bolus injection (18 mg/m^2), measured after the first administration, were plotted as a function of time (Fig. 1). These curves show a rapid initial decline of CMM and a fast appearance of CMMOH with a relatively slow disappearance, resulting in higher plasma concentrations of CMMOH than of CMM after 2–4 hr.

When incubated with whole blood at 37°C the concentration of CMM in plasma declined to 50% of the initial value (4×10^{-7} M) within 5 min, followed by a decline to 32% after 1 hr. This loss of drug in the plasma may contribute significantly to the first rapid distribution phase (Fig. 1). It indicates that in those samples taken 5 min following drug administration, the plasma concentration is more reliable, due to the established equilibrium between plasma and erythrocytes, than the very first samples, for which variations in handling in the clinic are more critical. In this incubation the CMMOH concentration in plasma came up to 10% of the initial concentration of CMM. This may at least partly explain the fast appearance of CMMOH in plasma.

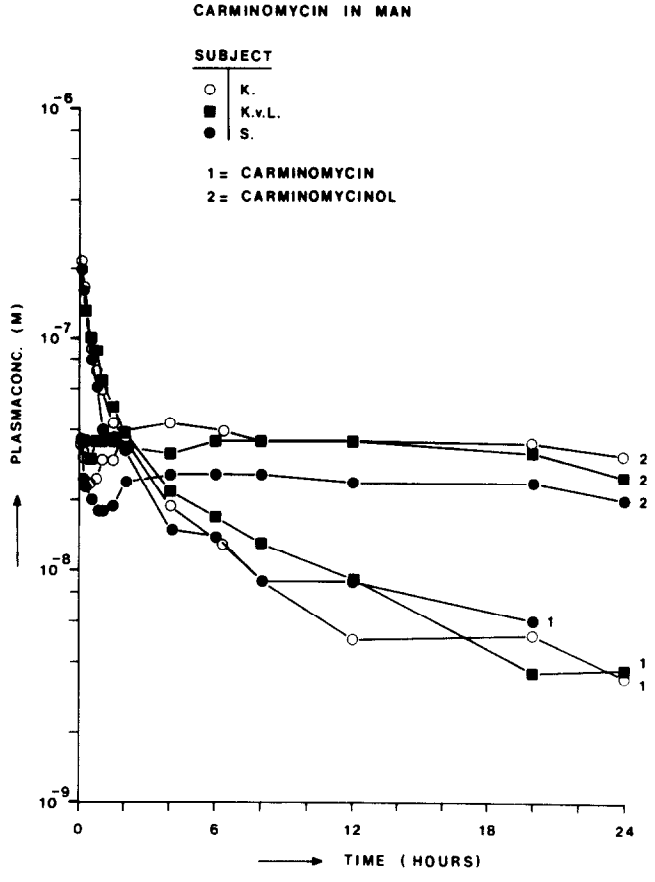
Figure 2 shows plasma concentrations for CMMOH during a more prolonged time period, showing a half-life of approximately two days. In an experimental tumor system of *in vitro* growing L1210 cells, a CMM concentration of CMMOH of 10^{-8} M caused a growth reduction of 30% in 24 hr. This may suggest cytotoxic activity coming from CMMOH in the plasma for several days.

When compared to adriamycinol, the metabolite after reduction of adriamycin [6], the plasma concentration ratio of CMMOH to CMM is significantly higher. This may be an important factor in explaining differences in clinical observations between both drugs.

For each patient the area under the plasma concentration vs time curve (AUC) for CMM and CMMOH was calculated for periods of 24 and 48 hr respectively (Table 1). For this purpose the trapezoidal rule was used [7]. The AUC was calculated for these periods rather than for the whole curve in order to avoid errors because of the relatively slow later disappearance phase.

The influence of the differences in the first steep disappearance phase of the different patients, which may be affected by variations in clinical handling of samples or administration time, on the measured AUC was 5% at most.

Values of the ratio of the AUC for CMMOH and CMM (patients T and B with lowest AUC's are excluded because of relatively high error) was fairly constant (3.1 ± 0.7, $n = 7$). This indicates no correlation between a lower AUC of CMM and a higher metabolism rate towards CMMOH, but rather a higher excretion or distribution affecting both compounds similarly. Clinical toxicity is also represented in Table 1, using the WHO grading. The cor-


CARMINOMYCIN IN MAN
SUBJECT
K.
K.v.L.
S.
1 = CARMINOMYCIN
2 = CARMINOMYCINOL
PLASMACONC. (M)
TIME (HOURS)

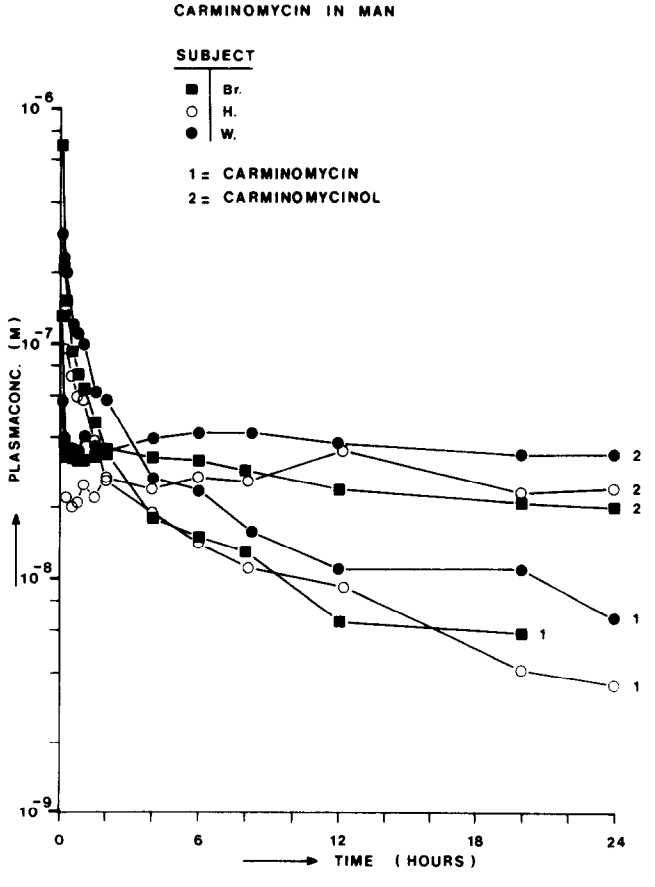

CARMINOMYCIN IN MAN
SUBJECT
Br.
H.
W.
1 = CARMINOMYCIN
2 = CARMINOMYCINOL
PLASMACONC. (M)
TIME (HOURS)

Fig. 1.

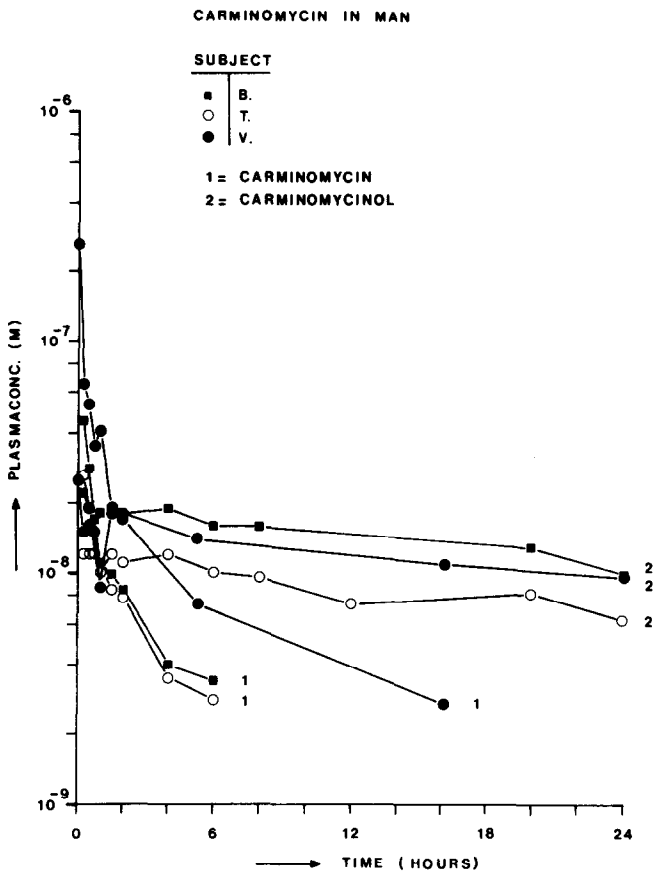


Fig. 1. Plasma concentration vs time curves for carminomycin and carminomycinol after i.v. bolus injection, dose 18 mg/m².

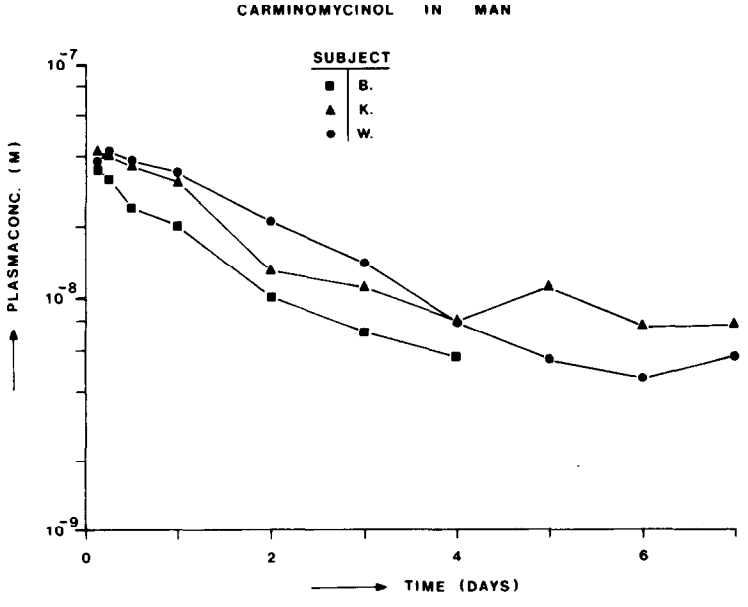


Fig. 2. Plasma concentrations of 3 patients of Fig. 1 followed for several days after administration.

Table 1. *Area under the curve (AUC, unit* 10^{-8} mol/l/hr) *of* 9 *patients (Fig.* 1) *vs clinical toxicity (WHO grades). Karnofsky performance status has been given after treatment*

Patient	AUC_{CMM}	AUC_{CMMOH}	Hematologic	Gastro-intestinal	Cardiac	Miscellaneous	Karnofsky	Total
V	18	30–50	2	0	0	0	(100)	2
T	4–7	34	3	0	0	1	(100)	4
B	5–8	45	2	0	0	1	(90)	3
S	32	93	3	2	0	1	(90)	6
Br	37	99	10	1	2	0	(50)	13
H	30	113	9	4	1	0	(70)	14
KvL	36	137	2	1	2	0	(100)	5
K	36	139	2	1	2	0	(30)	5
W	57	158	7	3	2	1	(50)	13

relation is especially notable for gastro-intestinal and heart toxicity. Two patients (Br and H) suffered severe bone marrow suppression and one (Br) died in a septic shock. This patient had in common with patient K a preterminal cardiac arrythmia. Both had been previously irradiated in the heart region and autopsies showed heart fibrosis.

CONCLUSIONS

High pressure liquid chromatography can be used to study pharmacokinetics of CMM and CMMOH in man. The observed correlation between the AUC and the absence of toxicity needs to be extended to a larger group of patients and should be investigated for other anthracyclines.

Toxicity of CMMOH has to be screened more in experimental systems. In case of less toxicity of CMMOH relative to CMM, a more frequent dose schedule, using lower doses of CMM in order to diminish CMM peak levels, may be advantageous.

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