Pharmacokinetics of Anthracyclines in Dogs: Evidence for Structure-Related Body Distribution and Reduction to Their Hydroxy Metabolites

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Abstract: The pharmacokinetic disposition of the anthracyclines, adriamycin (doxorubicin), daunorubicin, 4'-epi-adriamycin, carminomycin, and 4-demethoxy-daunorubicin, and the formation of their reduced C_{13} hydroxy metabolites were studied in dogs. The presence of a C_{14} hydroxy group (adriamycin and 4'epi-adriamycin) drastically reduces the appearance of the C_{13} hydroxy metabolites in plasma. Substitution of the C_4-H with C_4-OH and C_4-OCH_3, in this rank order, decreases the area under the plasma concentration-time curves of the parent compounds and their C_{13} hydroxy metabolites.

Studies on the structure-activity relationship among the anthracyclines have resulted in a number of promising cytotoxic drugs such as adriamycin (= doxorubicin, ADM), daunorubicin (DAR), carminomycin (CAM), 4'-epi-adriamycin (4'-epi-ADM), and 4-demethoxy-daunorubicin (4-dem-DAR). Clinical trials have failed to reveal clear criteria for the selection of the superior antineoplastic agent among these compounds (1–10). The five congeners studied here differ in their chemical structure at the positions C_4 and C_{14} of the anthraquinone chromophore and at the $C_{4'}$ position of the daunosamine sugar moiety (Fig. 1).

ADM and DAR are metabolized to a large extent (11, 12). One major metabolic pathway involves reductive cleavage of the sugar moiety to yield the anthraquinone aglycones. The other important known metabolic pathway is the reduction of the C_{13} carbonyl function in the C_9 side chain to give the corresponding C_{13} hydroxy metabolites (such as adriamycinol, ADM-ol; see Fig. 1) which also possess antitumor activity (13).

ADM and DAR are mainly eliminated via the bile (14). Only a minor fraction of the administered dose (10 % or less) is excreted by the kidneys (15, 16).

The purpose of this investigation was to compare the pharmacokinetic properties of the five anthracyclines and their corresponding C_{13} hydroxy metabolites. Dogs were selected as the experimental animal, since such a comparative pharmacokinetic study is not readily feasible in patients. The effects of chemical alterations of the anthracycline drugs on their overall pharmacokinetic disposition and on the formation of reduced C_{13} hydroxy metabolites were determined by measuring the plasma concentration-time curves and renal excretion of parent drugs and their respective metabolites.

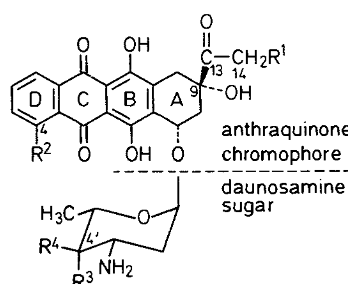

Fig. 1 Structural variations of five anthracycline drugs. Reduction of the C_{13} carbonyl to C_{13} hydroxy leads to the corresponding metabolites, i. e., ADM-ol, 4'-epi-ADM-ol, DAR-ol, CAM-ol, and 4-dem-DAR-ol.

		R^1	R^2	R^3	R^4
adriamycin	(ADM)	OH	OCH_3	OH	H
4'-epi-adriamycin	(4'-epi-ADM)	OH	OCH_3	H	OH
daunorubicin	(DAR)	H	OCH_3	OH	H
carminomycin	(CAM)	H	OH	OH	H
4-demethoxy-daunorubicin	(4-dem-DAR)	H	H	OH	H

Materials and Methods

Drugs and Chemicals

4-dem-DAR, adriamycinol (ADM-ol), and adriamycinone were supplied by Farmitalia (Milano, Italy). All other drugs were commercially available for clinical application and were obtained from: Roger Bellon (Neuilly, France), ADM; Specia (Paris, France), DAR; Bristol Meyers (USA), CAM; Farmitalia (Milano, Italy), 4'-epi-ADM. All other chemicals were of analytical grade and were used without further purification.

Drug Analysis

A reversed phase high performance liquid chromatographic method was used to determine the concentrations of parent compounds and hydroxy metabolites in plasma and urine (17). Other metabolites, such as adriamycinone cannot be analyzed by this method.

From spiked plasma samples a calibration curve in the anticipated concentration range of the samples was prepared each

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morning before analysis. During the day the analytical equipment was checked by injection of spiked plasma samples. The calibration curves went through zero with correlation coefficients always better than 0.98. The coefficient of variation of the assay for concentrations of 1 ng/ml of the parent drug was 10 % (n = 5).

Eluent. The eluent of the analytical column consisted of acetonitrile and 0.01 M H_3PO_4 in demineralized water. All anthracyclines could be measured with only slight variations in the acetonitrile concentration; ADM, 4'-epi-ADM, 30 % v/v; DAR, CAM, and 4-dem-DAR, 45 % v/v.

Detection. A fluorescent detector (Perkin Elmer, model 3000) equipped with a red-sensitive photomultiplier was used at $\lambda_{exc.}$ = 474 nm. Alterations in the electron density in the D-ring in Figure 1 change the maximum emission wavelength from 590 nm to 568 nm for 4-dem-DAR and to 555 nm for CAM. The detection limits are 0.5 ng/ml for all parent compounds and their hydroxy metabolites in plasma at a signal to noise ratio of 3.

Recoveries. The recoveries after the extraction procedure from plasma of the parent compounds and their hydroxy metabolites, performed in triplicate for concentrations ranging from 5 to 500 ng/ml are about 80 ± 3 % (mean ± SD) (17). The hydroxy metabolites of 4'-epi-ADM, CAM, and 4-dem-DAR were not available as pure substances. The plasma concentration profile of the hydroxy metabolite of DAR was measured to be identical to the one reported in literature (18). Because of the similarity of the chromatograms of plasma and urine samples after administration of CAM, 4'-epi-ADM, and 4-dem-DAR to those obtained after administration of ADM and DAR, and because of the similarity of the plasma concentration-time and renal excretion rate-time profiles, it was assumed that the compounds with the shorter retention-time on the column are identical to the C_{13} hydroxy metabolites. To determine the recoveries of the C_{13} hydroxy metabolites of DAR, 4'-epi-ADM, CAM, and 4-dem-DAR, a predetermined amount of dog's urine containing a high concentration of the C_{13} hydroxy metabolite was used to spike blank plasma. The area under the peak of the chromatogram of the urine, which can be injected directly, is compared with the area under the peak of the extracted spiked plasma. Assuming that the emission coefficients are the same for parent compound and metabolite, the concentration of the metabolites can be determined with reasonable accuracy. In the case of ADM and its reduced metabolite ADM-ol, the emission coefficients are the same.

Dog Studies

The anthracyclines were randomly administered as a single i.v. bolus injection with an interval of 4 weeks between each injection to two beagle dogs, bred by the Central Animal Laboratory of the University of Nijmegen (The Netherlands). Body weight: 9.5 kg (dog nr. 617), 10.5 kg (dog nr. 604). An additional dog (nr. 499) with a body weight of 10.2 kg was injected ADM and its C_{13} hydroxy metabolite ADM-ol. During the first 8 h after drug administration, the dogs were kept anesthesized with pentothal. Thereafter, they were placed into metabolic cages for collection of spontaneously voided urine.

Blood and Urine Samples

At 0.05, 0.15, 0.30, 0.45, 1.00, 1.30, 2.00, 3.00, 4.00, 5.00, 6.00, and 7.00 h, blood samples were taken from an indwelling venous catheter in the front leg into polypropylene tubes, containing heparin. Samples at 13.00, 23.00, 27.00, and 31.00 h were taken by venipuncture. The samples were immediately centrifuged (1500 g). Urine samples were obtained from a bladder catheter during the period of anesthesia at regular time intervals. One additional urine sample was collected 24 h after drug administration from the dogs in the metabolic cages. The plasma and urine samples were kept frozen (−20° C) until analysis.

Curve Fitting and Calculations

Curve fittings and calculations of the pharmacokinetic parameters were performed with standard statistical procedures on a HP 9810 calculator (Hewlett Packard). The plasma concentration-time profiles of the parent drugs were described by the sum of three exponentials:

$$C_t = A\,e^{-\alpha t} + B\,e^{-\beta t} + C\,e^{-\gamma t}$$

The intercepts on the concentration axes A, B, and C and the corresponding exponents α, β, and γ were obtained using the stripping procedure. The area under the plasma concentration-time curve of the parent compound (AUC_p), the volume of the central compartment (V_1), the apparent distribution volume (V_f), and the total body clearance (Cl_p) were calculated with the aid of the following equations (19):

$$V_1 = D/(A+B+C)$$
$$AUC = A/\alpha+B/\beta+C/\gamma$$
$$V_f = D/(A/\alpha^2+B/\beta^2+C/\gamma^2)/AUC^2$$
$$Cl_p = D/AUC$$

The area under the curve of the metabolite (AUC_m) was calculated using the trapezoidal rule. The renal clearances of the various compounds were calculated starting from 2.5 h after administration.

Because of a lag-time, the first samples did not fit the linear equation:

$dQ/dt = C_p \times Cl_{r(p,m)}$ in which:
dQ/dt = renal excretion rate (ng/min)
C_p = plasma concentration (ng/ml)
Cl_{rp} = renal clearance of parent compound (ml/min)
Cl_{rm} = renal clearance of metabolite (ml/min)

Results

Figures 2 through 6 show the renal excretion rates and the plasma concentration-time profiles of both parent compounds and C_{13} hydroxy metabolites after administration of the five anthracyclines in dog nr. 604. The pharmacokinetic parameters obtained from dogs nr. 604 and nr. 617 are listed in Table 1.

Structural Variation in R_1(-OH,-H)

ADM (Fig. 2) differs from DAR (Fig. 3) by the presence of a C_{14}-OH group versus a proton (C_{14}-H). The change from -OH to -H results in a increased concentration of the C_{13} hydroxy metabolite in plasma. The AUC_m of ADM-ol is 94, and that of DAR-ol 476 μg.h/l, whereas the AUC_p of the parent compound is decreased, 288 for ADM and 86 μg.h/l for DAR. These differences are also expressed in the following pharmacokinetic parameters: V_1, 19.1 versus 51.0 l; V_f, 798 versus 2537 l; Cl_p, 607 versus 2031 ml/min, for ADM and DAR, respectively. The renal clearance of the parent compound (Cl_{rp}) is increased from 55 to 134 ml/min, and that of the metabolite (Cl_{rm}) from 15 to 59 ml/min. The recovery

Table 1. Pharmacokinetic Parameters of the Anthracyclines in Dog after i.v. Bolus Injection[a,b]

Drug	dog	A ng/ml	B ng/ml	C ng/ml	$t_{1/2\alpha}$ min	$t_{1/2\beta}$ h	$t_{1/2\gamma}$ h	V_1 l	V_f l	Cl_p ml/min	Cl_{rp} ml/min mean ±SD	Cl_{rm} ml/min mean ±SD	AUC_p µg.h/l	AUC_m µg.h/l	AUC_m/AUC_p
ADM	604	531	10.9	6.4	5.4	1.12	21.5	19.1	798	607	55 ± 20	15 ± 3	288	94	0.33
ADM	617	789	28.8	11.2	5.9	0.82	30.7	10.4	641	426	47 ± 20	19 ± 6	333	107	0.32
4'-epi-ADM	604	125	9.2	4.0	7.1	2.11	40.0	71.9	1698	589	59 ± 31	12 ± 8	280	131	0.47
4'-epi-ADM	617	100	15.3	3.7	8.0	1.80	35.7	84.1	1584	662	50 ± 25	26 ± 10	252	154	0.61
DAR	604	198	6.0	1.5	5.3	1.13	24.2	51.0	2537	2031	134 ± 17	59 ± 11	86	476	5.53
DAR	617	74	3.8	1.7	5.8	1.29	19.3	107.2	2679	2163	62 ± 30	67 ± 35	66	280	4.27
CAM	604	92	10.7	3.9	20.3	1.13	12.1	98.5	782	1358	12 ± 2	7 ± 1	129	698	5.42
CAM	617	47	20.5	2.3	20.5	1.33	15.8	122.4	829	1233	9 ± 5	9 ± 5	115	898	7.81
4-dem-DAR	604	330	14.9	4.7	6.0	2.10	18.9	34.5	540	711	6 ± 3	6 ± 3	305	1893	6.22
4-dem-DAR	617	249	45.1	5.7	7.1	1.65	20.4	33.4	517	601	4 ± 2	8 ± 3	319	1786	5.59

[a] The parameters were corrected for the dosage used, which was set to 1 mg/kg.
[b] For explanation of the symbols used, see text.

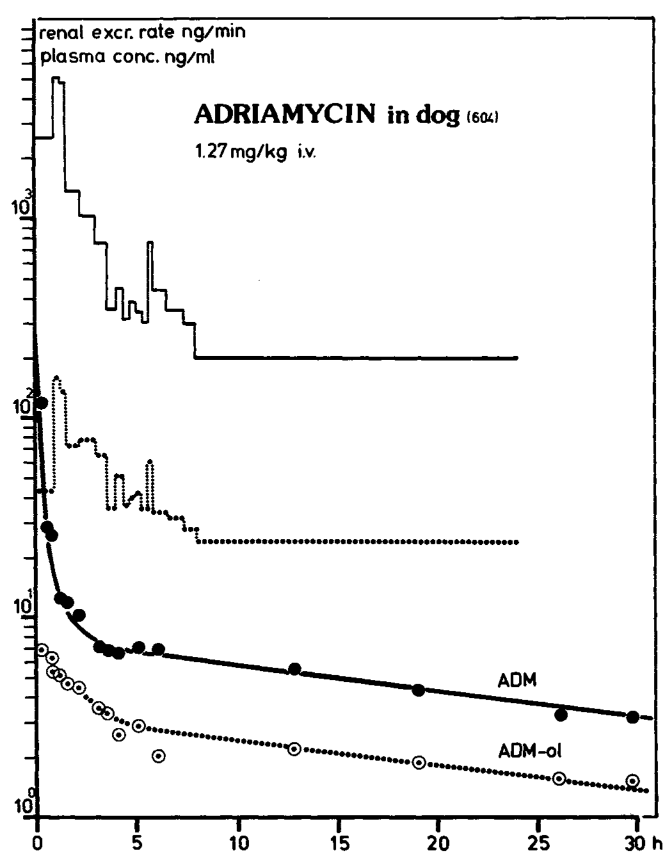

Fig. 2 Plasma concentration and renal excretion rate time profiles of adriamycin (ADM) and adriamycinol (ADM-ol) in dog 604 after an i.v. bolus injection of 1.27 mg/kg.

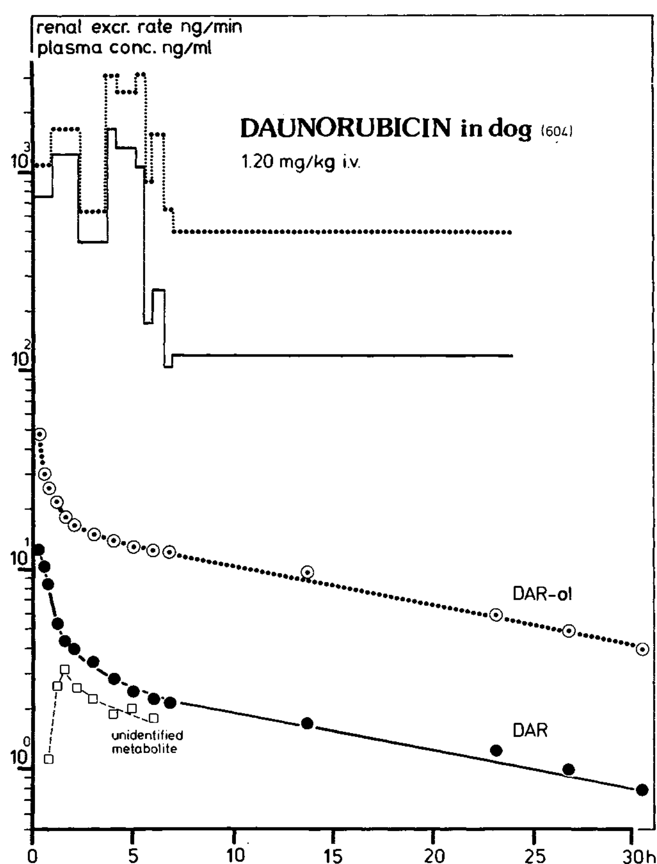

Fig. 3 Plasma concentration and renal excretion rate time profiles of daunorubicin (DAR) and daunorubicinol (DAR-ol) in dog 604 after an i.v. bolus injection of 1.20 mg/kg.

of unchanged drug in the urine over 24 h accounts for 5.2 % and 2.7 % of the administered dose, and 0.13 % and 8.0 % are found as the C_{13} hydroxy metabolites for ADM and DAR, respectively.

Structural Variation in R_2 (-OCH_3,-OH,-H)

The structural differences among the anthracyclines DAR (Fig. 3), CAM (Fig. 4), and 4-dem-DAR (Fig. 5) are located at the C_4 position. The renal clearances of the parent compounds are 134, 12, and 6 ml/min; those of the hydroxy metabolites are 59, 7, and 6 ml/min. Over a period of 24 h, the recovery from urine of unchanged drug is 2.7 %, 0.54 %, and 0.60 %; the values for the respective C_{13} hydroxy metabolites are 8 %, 2.5 %, and 3.1 % for DAR, CAM, and 4-dem-DAR, respectively. The AUC_ps of the parent compounds are 86, 129, and 305 µg.h/l; those of the metabolites are 476, 698, and 1893 µg.h/l. As can be seen from Table 1, the calculated pharmacokinetic parameters V_1, V_f, $t_{1/2}$, Cl_p,

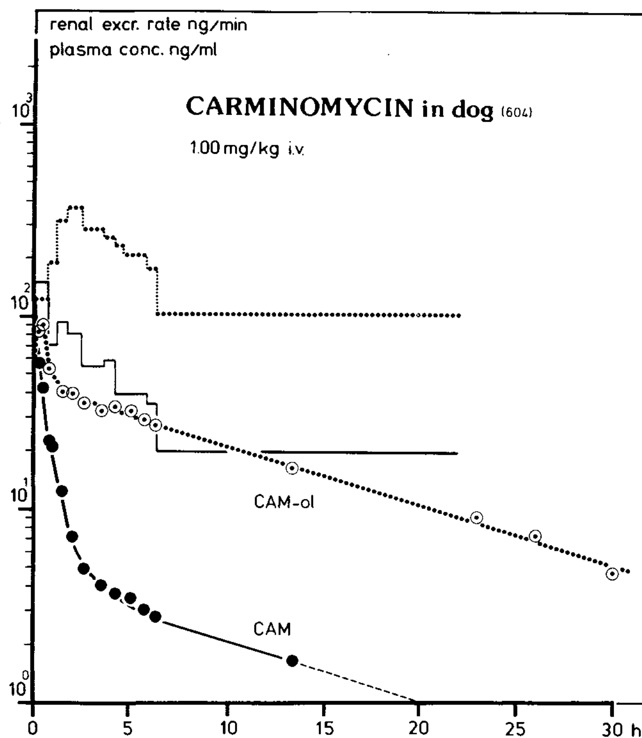

Fig. 4 Plasma concentration and renal excretion rate time profiles of carminomycin (CAM) and carminomycinol (CAM-ol) in dog 604 after an i.v. bolus injection of 1.00 mg/kg.

and A also show large differences. The unidentified metabolite, shown in Figure 3, was not detected after the administration of CAM and 4-dem-DAR.

Structural Variation in R_3, R_4

The variation in structure at the $C_{4'}$ position in the daunosamine sugar of ADM and 4'-epi-ADM is responsible for only minor differences in their pharmacokinetic profiles (Figs. 2 and 6). The renal clearance of ADM and 4'-epi-ADM are 55 and 59 ml/min and those of the C_{13} hydroxy metabolites 15 and 12 ml/min, respectively. The recovery from urine is 5.2 % and 5.6 % for the parent compounds and 0.13 % and 0.37 % for the C_{13} hydroxy metabolites. The AUC_p's of the parent compounds are practically the same, 288 and 280 µg.h/l. The AUC_m of the metabolite of 4'-epi-ADM is higher than that of ADM-ol, 131 versus 94 µg.h/l. The other calculated pharmacokinetic parameters show larger differences. The intercept A changes from 531 to 125 ng/ml, $t_{1/2}$ from 21.5 to 40 h, V_1 from 19.1 to 71.9 l, and V_f from 798 to 1698 l, for ADM and 4'-epi-ADM, respectively.

Figures 2 through 6 show a high concentration of the C_{13} hydroxy metabolite in the first blood sample taken after injection (5 min). Thereafter, the concentration-time curve of the metabolite declines parallel to the curve of the parent compound. When the metabolite adriamycinol (ADM-ol) is administered (Fig. 7), the $t_{1/2}$ is eight times lower compared with the measured $t_{1/2}$ after the administration of ADM

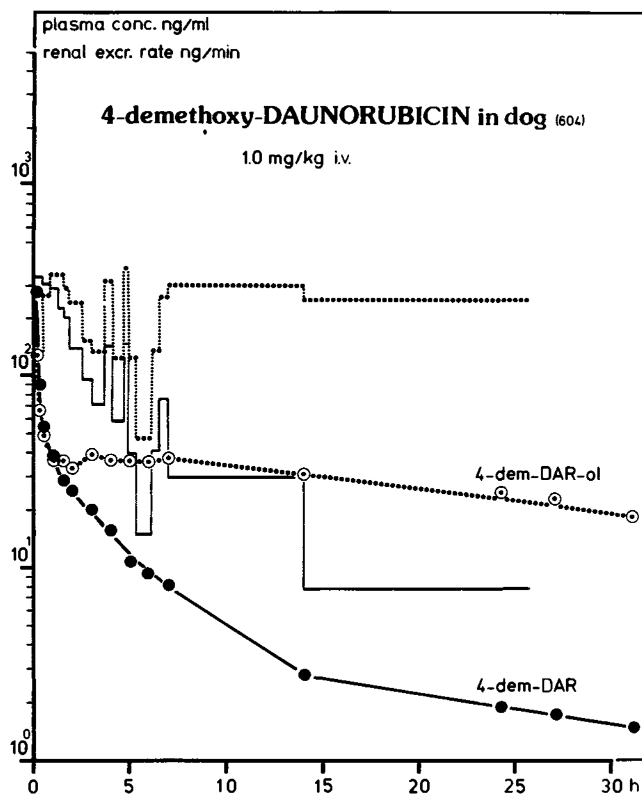

Fig. 5 Plasma concentration and renal excretion rate time profiles of 4-demethoxy-daunorubicin (4-dem-DAR) and 4-demethoxy-daunorubicinol (4-dem-DAR-ol) in dog 604 after an i.v. bolus injection of 1.00 mg/kg.

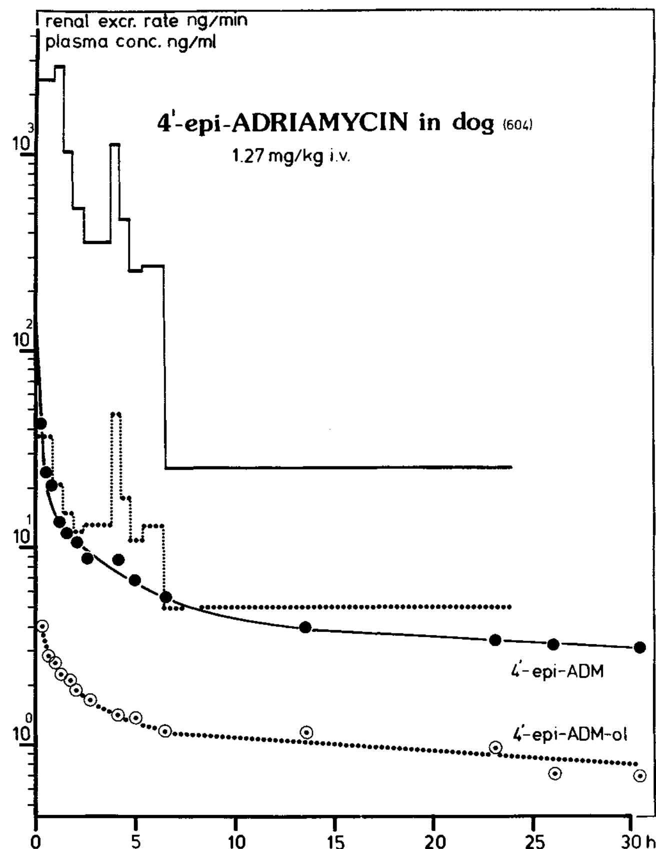

Fig. 6 Plasma concentration and renal excretion rate time profiles of 4'-epi-adriamycin (4'-epi-ADM) and 4'-epi-adriamycinol (4'-epi-ADM-ol) in dog 604 after an i.v. bolus injection of 1.27 mg/kg.

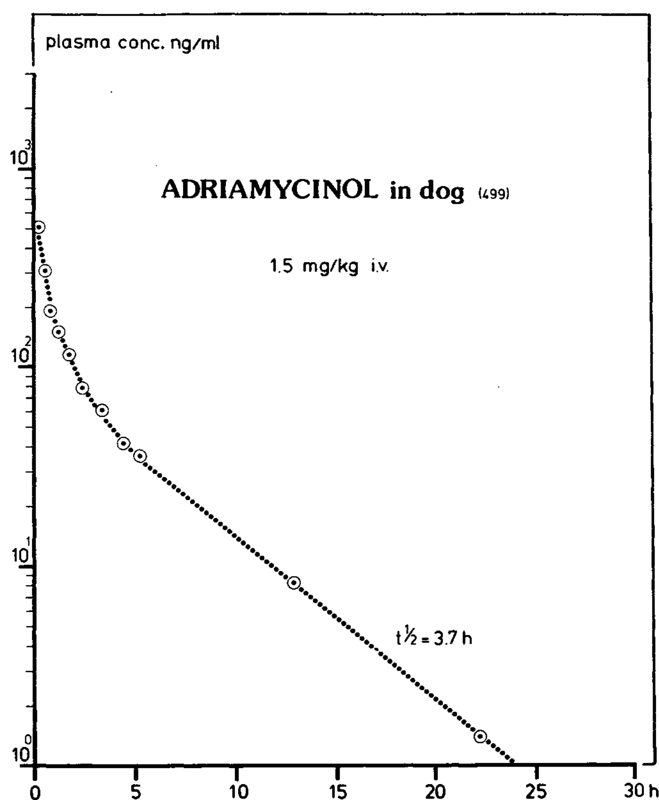

Fig. 7 Plasma concentration time profile of adriamycinol (ADM-ol) after the administration of 1.5 mg/kg of adriamycinol in dog 499 as i.v. bolus injection. The intrinsic $t_{1/2}$ of ADM-ol is 3.7 h. Adriamycin (ADM) plasma concentrations were below defectable levels.

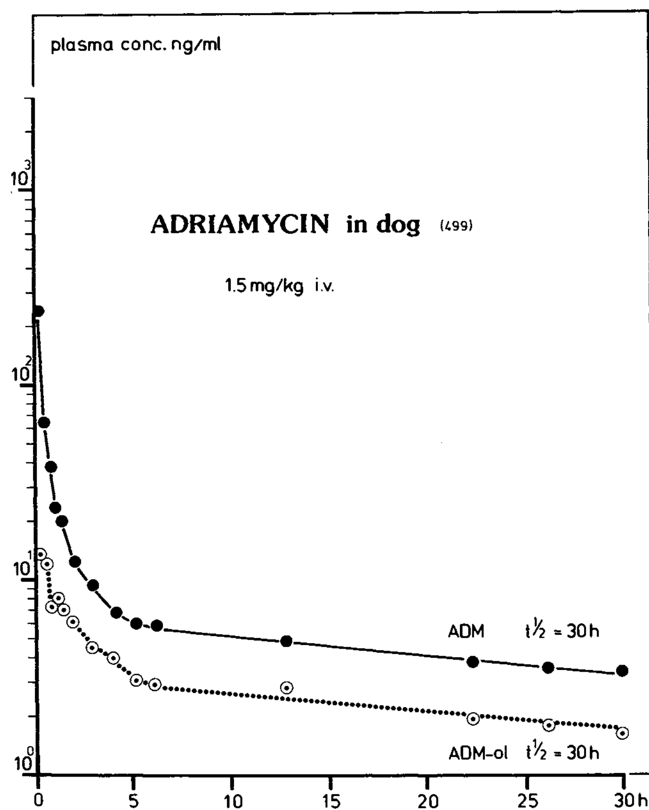

Fig. 8 Plasma concentration time profiles and adriamycin (ADM) and adriamycinol (ADM-ol) after the administration of 1.5 mg/kg ADM as i.v. bolus injection in dog 499. The measured $t_{1/2}$ of both parent compound and metabolite is 30 h.

(Fig. 8). The apparent $t_{1/2}$ of ADM-ol is 30 h, the intrinsic $t_{1/2}$ is 3.7 h, as measured in dog nr. 499. After the administration of ADM-ol, the presence of ADM was not demonstrated.

Discussion

Structural Variation in R_1

The structural variation at the C_{14} position, that is, -H versus -OH, results in a large difference in the plasma concentration of the hydroxy metabolite. When the ratios of the AUC_m of the metabolite and the AUC_p of the parent compound (AUC_m/AUC_p) for ADM and DAR are compared, a rise from 0.33 for ADM to 5.53 for DAR can be observed. For CAM and 4-dem-DAR, which have different substituents in R_2 in addition to the proton in R_1, these values increase to 5.42 and 6.22. This suggests that the other substituents in R_2 are too distant from the reaction center to affect the reduction. The same holds true when the position of the -OH group is changed at C_4', as in the case of 4'-epi-ADM. Its AUC_m/AUC_p ratio of 0.47 is comparable with that of ADM, 0.33.

The following factors may account for the more than tenfold increase in the ratios of AUC_m/AUC_p for DAR, CAM, and 4-dem-DAR compared with those ADM and 4'-epi-ADM:

1. In the case of R_1 = -OH (ADM and 4'-epi-ADM) a different metabolic pathway is preferred. Unknown metabolites, in addition to those already identified, that is aglycones, glucuronides, etc. (12), may have been generated. Failure to detect such metabolites could have be caused by:
   (a) loss of fluorescent properties or a large change in the fluorescent maximum compared with the parent compound, which may result from metabolic changes of the fluorescent anthraquinone chromophore;
   (b) distribution of the metabolite over a large volume, so that concentrations will stay below the detection limit of the analytical procedure.
2. Increased metabolic reactivity or biliary excretion of the C_{13} hydroxy metabolites occurs when the C_{14} position is hydroxylated, (R_1 = -OH) resulting in a lower AUC_m of the metabolite.
3. The apparent higher AUC_m values of the hydroxy metabolites may be accounted for in part by a smaller volume of tissue distribution of the metabolites, when R_1 = -H.
4. Reduction of the parent compound is more important in the case of the R_1 = -H derivatives as a result of an increased availability of the parent compound, that is lower tissue or protein binding, or an increased affinity to the reducing enzymes.

Structural Variation in R_2 (-OCH_3,-OH,-H)

In the case of DAR, CAM, and 4-dem-DAR, where structural variations are located at the C_4 position (R_2), the AUC_m/AUC_p ratios are approximately the same, i.e., 5.53, 5.42 and 6.22, respectively. The AUC_p's of the parent compounds increase from 86 to 129 and to 305 µg.h/l. These results indicate that the AUC_m of the metabolite correlates with the AUC_p of the parent compound. The effect of the variations at C_4 may be caused by distribution differences. However, no correlation can be observed between the

AUC_p's of the parent compounds and the partition coefficients, obtained from an octanol-water system (0.46, 1.73, and 1.68, respectively). The differences in electron density of the D-ring, caused by the different substituents, may also contribute to the observed differences in the AUC's as a result of altered protein binding or metabolic pathways.

Structural Variations in R_3, R_4

The position of the -OH group at C_4, seems to have some influence on the ration AUC_m/AUC_p, which is 0.33 in the case of ADM and 0.47 in the case of 4'-epi-ADM. However, as the study was only performed in two dogs, a conclusion about the significance of this small difference is uncertain.

Renal Clearances

Two further pharmacokinetic parameters, in addition to the AUC', were measured, that is, the renal clearances of the parent compounds (Cl_{rp}) and their C_{13} hydroxy metabolites (Cl_{rm}). Although these values were not corrected for protein binding, comparison of the results among these closely related congeners can reveal useful information. The renal clearances of ADM and 4'-epi-ADM (55 and 59 ml/min) and their reduced metabolites (15 and 12 ml/min) are similar. Therefore, it can be surmised that a change of the C_4-OH configuration has little effect on protein binding and renal excretion.

In the case of DAR, CAM and 4-dem-DAR, there are large differences among the renal clearances of the parent compounds (Cl_{rp}: 100, 11, and 5 ml/min, respectively). Similar changes were observed for the renal clearances of the C_{13} hydroxy metabolites of DAR, CAM, and 4-dem-DAR (Cl_{rm}: 64, 8, and 7 ml/min). Whether differences in protein binding or tubular reabsorption are responsible still needs to be examined. Neither urinary flow nor pH dependency of the renal clearance of the anthracyclines and their metabolites was detectable.

Elimination Half-Life of the C_{13} Hydroxy Metabolites

Figures 2 through 6 show that the hydroxy metabolites are already present in high concentrations in the first sample taken after injection. Thereafter, the concentration of the metabolite declines parallel to that of the parent compound. On the basis of the results shown in Figures 7 and 8, it can be concluded that the intrinsic $t_{1/2}$ of ADM-ol is much smaller than the apparent $t_{1/2}$ of ADM-ol after the administration of ADM (intrinsic $t_{1/2}$ = 3.7 h, apparent $t_{1/2}$ = 30 h). This phenomenon can usually be observed with metabolic equilibrium reactions (20). However, after the administration of ADM-ol, no ADM was detectable. It can therefore be concluded that the metabolic formation represents the slow, rate-limiting step in the kinetic disposition of ADM-ol in the body. Hence, ADM and ADM-ol plasma level profiles are parallel. Whether this phenomenon also applies to the other anthracyclines studied here remains to be examined by injecting the chemically synthesized C_{13} hydroxy metabolites to determine their intrinsic pharmacokinetic parameters. Furthermore, complete metabolic pathways must be elucidated for each anthracycline.

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