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Pharmacokinetics of doxorubicin and its metabolite doxorubicinol in rabbits with induced acid and alkaline urine

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Summary. *The pharmacokinetics of doxorubicin in rabbits preloaded either with ammonium chloride or sodium hydrogencarbonate have been investigated following single IV administration of 5 mg/kg.*

Plasma samples and urine collections were obtained over 3 h following administration, and were assayed in duplicate for doxorubicin and its main metabolite doxorubicinol by reversed-phase high-pressure liquid chromatography.

The plasma concentration of doxorubicin was fitted to an open two-compartment model.

The areas under the plasma concentration-time curves (AUC) of doxorubicin in rabbits with alkaline urine were approximately half the areas in rabbits with acid urine. A pharmacokinetic analysis indicated an increase in the central volume of distribution, which is interpreted as an increase in tissue permeability in the alkaline state, due to the acid-base properties of the doxorubicin molecule.

The renal excretion of doxorubicin and doxorubicinol was quantitatively similar in the two groups of rabbits. The total renal excretion of anthracyclines during the experiment was calculated to approximately 6% of the administered dose. The clearances of doxorubicin were initially three times higher than inulin clearance, but approximated this value at the end of the experiment.

The renal handling of doxorubicin in the rabbit is explained by glomerular filtration followed by tubular secretion and finally by a reabsorption mechanism with limited capacity.

Introduction

Doxorubicin (Adriamycin) is an anthracycline antibiotic widely used in cancer chemotherapy. The molecule consists of an aromatic aglycone linked to the amino sugar daunosamine [1].

Doxorubicin is partially ionized under physiological conditions by protonation of the amino group, the pK_a being 7.2–7.6 [5, 8].

The transport of doxorubicin and daunorubicin across cell membranes has been shown to be pH-dependent: Raising pH from 6.5 to 7.5 results in a six-fold increase in the transport rate [6, 13].

Therefore it seemed likely that differences in acid-base balance would alter the pharmacokinetics of doxorubicin. The aims of the present investigation were to study the pharmacokinetics of doxorubicin under different acid-base conditions, firstly with reference to the plasma concentration-time curve, and secondly with reference to the renal handling of doxorubicin.

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Materials and methods

The experiments were performed in male Danish breed white rabbits weighing 2.0–2.5 kg. One hour before the experiments the rabbits were given either 50 ml tap water (experiments with alkaline urine) or 50 ml of a 0.8% NH_4Cl solution (experiments with acid urine) by stomach tube. The animals were anesthetized with urethane, 1.5 g/kg (in 25% solution). Preloading was achieved by continuous infusion into the left ear vein of a solution containing 0.3% NaCl and 1.3% glucose at 1 ml/min.

The right external jugular vein was cannulated with a polyethylene tube for blood sampling and the trachea was cannulated to ensure free respiration. Urine was collected by way of a vesicourethral catheter.

The plasma concentration of doxorubicin was determined at 10, 30, 50, 70, 100, and 150 min (2–3 ml blood with heparin as anticoagulant). Urine was collected during four periods of 20 min, one of 40 min, and one of 60 min.

The inulin clearance was determined at three time points, just before doxorubicin administration, halfway through the experiment, and at the end.

Approximately 1 h before the administration of doxorubicin a priming dose of 100 mg inulin (10 ml 1% inulin in 0.9% NaCl solution) was infused into the left ear vein followed by a continuous infusion of inulin (2.5 mg/min) in a solution similar to that used for preloading. To obtain acid urine 0.4% NH_4Cl was added to the infusion solution and to obtain alkaline urine 0.6% $NaHCO_3$ was added. The experiments started with a 20-min period of urine collection for determination of inulin clearance. For this purpose a 2- to 3-ml blood sample was taken in the middle of the period. Following this period doxorubicin 5 mg/kg was injected over 4 min into the left ear vein (2.5 mg/ml in 0.9% NaCl).

Urine was collected in periods of 20–60 min following the administration of doxorubicin and 2- to 3-ml blood sample was taken at the middle of each period.

Analytical methods. The plasma concentrations of doxorubicin and metabolites were determined by means of an HPLC method previously described [11]. The recovery of doxorubicin and doxorubicinol from rabbit plasma was 85% and 80%, respectively.

Table 1. Pharmacokinetic parameters obtained by applying a two-compartment open model to the plasma concentration-time data of doxorubicin (mean ± SEM, $n = 4$)

	Acidic	Alkaline	
Primary parameters			
A µg/ml	6.53 ± 0.94	1.92 ± 0.34	$P < 0.05$
α min^{-1}	0.159 ± 0.005	0.142 ± 0.016	NS
B µg/ml	0.174 ± 0.009	0.157 ± 0.009	NS
p min^{-1}	0.0054 ± 0.0002	0.0066 ± 0.0003	$P < 0.05$
Derived parameters			
AUC µg/ml · min	73.0 ± 7.0	36.9 ± 2.1	$P < 0.05$
V_1 l/kg	0.79 ± 0.11	2.65 ± 0.50	$P < 0.05$
Cl_B ml · min^{-1} · kg^{-1}	70.7 ± 7.7	136.6 ± 7.2	$P < 0.05$
k_{21} min^{-1}	0.0096 ± 0.0004	0.0172 ± 0.0008	$P < 0.05$
k_e min^{-1}	0.091 ± 0.005	0.056 ± 0.008	$P < 0.05$
k_{12} min^{-1}	0.064 ± 0.002	0.074 ± 0.009	NS

The concentration of doxorubicin and metabolites in urine was analyzed within 1 week with the same HPLC method as for plasma. For this, 3–4 ml urine was adjusted to pH 2 (1.9–2.1) with concentrated phosphoric acid and 1.00 ml of the resultant solution was diluted 1 : 10 or 1 : 5 with mobile phase (acetonitrile: 0.015 *M* phosphoric acid 35 : 65) depending on the doxorubicin concentration; 100 µl was injected into the liquid chromatograph. All plasma and urine samples were analyzed in duplicate. Doxorubicin, doxorubicinol, and doxorubicinone dissolved in mobile phase were used as standards. After adjustment of pH and dilution of the urine, samples could be kept for at least 1 month at +4° C. The collection of urine was performed in dimmed light because of the photolytic degradation of the anthracyclines [14].

The concentration of inulin in plasma was determined as described by Heyrovsky [10]. No interference was found between doxorubicin and inulin.

Pharmacokinetic calculations. A two-compartment open model was fitted to the data, with standard correction for the infusion time of 4 min [9]. The parameters in the equation: $A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t)$ were calculated for each animal by a Gauss-Newton procedure using the first derivatives of the equation with respect to the parameters. The calculations were worked on an RC-8000 computer.

Differences were analyzed by the two-sample Student's *t*-test. The results are expressed as the mean ± SEM.

Results

The plasma concentrations of doxorubicin and doxorubicinol in rabbits with acid and alkaline urine are shown in Fig. 1A and B, respectively. Statistical significance is reached between the doxorubicin plasma concentration at 10 min ($P < 0.002$), 30 min ($P < 0.05$), and 100 min ($P < 0.05$). The results of the pharmacokinetic analysis are shown in Table 1. The plasma concentrations of doxorubicinol in the alkaline state show the characteristics of a two-compartment model, but calculations of the parameters of this model cannot be applied because of the limited number of experimental points. The areas under the plasma concentration-time curves (AUC) for doxorubicinol were therefore calculated by the trapezoidal method and are: 5.3 ± 1.1 µg/ml × min and 5.2 ± 0.7 µg/ml × min for the acidic and alkaline state, respectively (not significant).

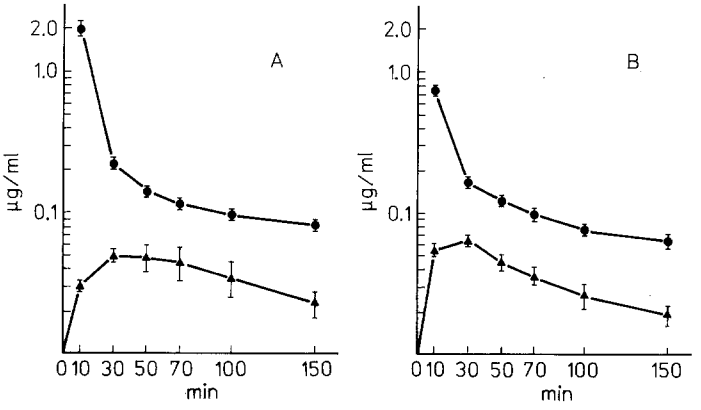

Fig. 1. Plasma concentrations of doxorubicin (●) and doxorubicinol (▲) in rabbits with acid (**A**) and alkaline (**B**) urine following IV administration of doxorubicin hydrochloride 5 mg/kg. Mean values ($n = 4$) and SEM are shown

Doxorubicin constitutes 75%–90% of the total amount of anthracyclines in plasma. Doxorubicinone could be detected neither in plasma nor in urine.

The renal excretion of doxorubicin and doxorubicinol during the 3-h experiment is shown in Table 2. The average renal excretion of doxorubicin in rabbits with acid urine was 5.2% ± 0.7% of the administered dose, in rabbits with alkaline urine, 4.4% ± 0.3% (NS). The corresponding percentages for doxorubicinol are 1.1% ± 0.4% and 1.5% ± 0.3%, respectively (NS). Doxorubicin therefore constitutes 65%–90% of the total amount of the two anthracyclines in urine. Two unidentified polar metabolites were detected in the urine, possibly conjugates, but constituing only a small part of the total amount of anthracyclines excreted in the urine.

The urine volume was twice as high in the acid group than in the alkaline group (155 ± 23 ml and 79 ± 15 ml, respectively; $P < 0.05$).

The inulin clearance was 7.7 ± 0.6 ml/min in the acid group and 10.0 ± 1.1 ml/min in the alkaline group ($P < 0.1$), and was constant during the observation period.

Urine pH in the two groups of rabbits was 6.0–6.5 and 7.8–8.2, respectively (Table 2).

The clearances of doxorubicin in the two groups of rabbits were calculated for each urine sample except for the first sampling period. The renal clearances of doxorubicin are shown in Fig. 2 together with the inulin clearances. Statistical

Table 2. Renal excretion[a] of doxorubicin (DOX) and doxorubicinol (DOXol) following administration of doxorubicin hydrochloride 5 mg/kg IV in rabbits with acid and alkaline urine

	0–20 min (μg)		20–40 min (μg)		40–60 min (μg)		60–80 min (μg)		80–120 min (μg)		120–180 min (μg)		Total excretion (% of dose)	
DOX	272 (64)	128 (26)	87 (10)	95 (14)	56 (5)	71 (10)	39 (2)	49 (6.2)	56 (6.4)	70 (4.3)	56[b] (3.7)	68[b] (3.2)	5.2 (0.7)	4.4 (0.3)
pH (mean)	6.4	8.0	6.4	7.9	6.3	8.0	6.3	8.2	6.2	8.1	6.2	8.2	Acid	Alkaline
DOXol	2.8 (0.7)	5.0 (1.7)	13 (4.3)	19 (5.3)	17 (5.8)	26 (7.8)	17 (5.6)	23 (5.7)	31 (10)	43 (11)	37 (13)	47 (9)	1.1 (0.4)	1.5 (0.3)

[a] Figures given are means with SEM in parentheses
[b] $P < 0.05$

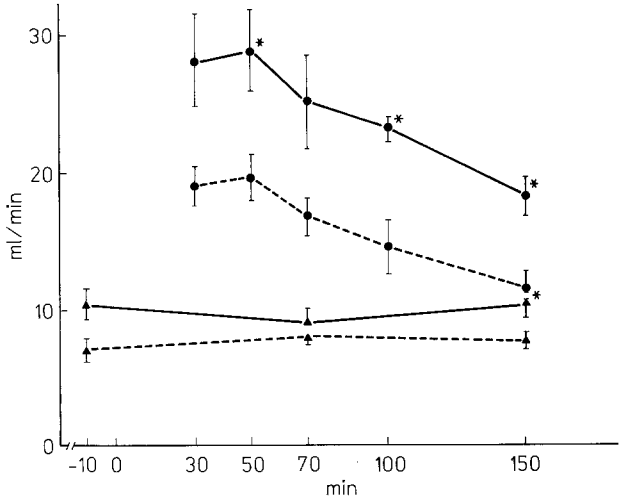

Fig. 2. Renal clearances of doxorubicin (●) and inulin (▲) in rabbits with acid (– – –) and alkaline (———) urine following IV administration of doxorubicin hydrochloride 5 mg/kg. Mean values and SEM are shown. Significant differences ($P < 0.05$) are marked with an asterisk

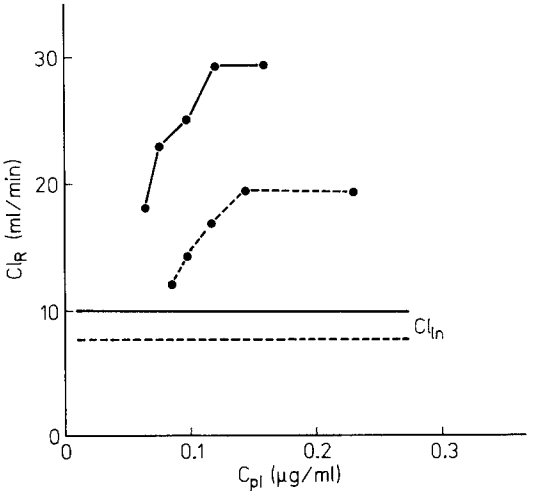

Fig. 3. Renal clearance of doxorubicin (ml/min) in relation to the plasma concentration (μg/ml) in rabbits with acid (– – –) and alkaline (———) urine following IV administration of doxorubicin hydrochloride 5 mg/kg. Each *point* represents the mean value from four animals (see Figs. 1 and 2). Cl_{In} is the mean inulin clearance in rabbits with acid (– – –) and alkaline (———) urine, respectively

significance was reached between the doxorubicin clearances at 40–60 min ($P < 0.05$), 80–120 min ($P < 0.01$), and 120–180 min ($P < 0.02$). When the doxorubicin clearances were expressed relative to the inulin clearances no statistical differences were found.

Figure 3 shows the renal clearance of doxorubicin, calculated as $\Delta X_R/\Delta t$, i.e., (rate of excretion)/$C_{pl,mean}$ (plasma concentration at the mid-point of the sampling interval). The result are calculated from the mean values for the four animals of each group. The inulin clearance measured is also shown in Fig. 3.

Discussion

The pharmacokinetics of doxorubicin and its main metabolite doxorubicinol were investigated in rabbits with induced acid and alkaline urine following IV administration of doxorubicin hydrochloride 5 mg/kg.

The plasma disappearance of doxorubicin and the formation of doxorubicinol are consistent with the results of other pharmacokinetic investigations in rabbits [2, 12, 15].

The pharmacokinetic analysis shows that the major difference in the plasma concentration decay curve of doxorubicin between rabbits with acid and those with alkaline urine is a difference in parameter A, which by standard kinetic calculations causes a 3-fold increase in the central volume of distribution in the alkaline compared with the acidic state.

This may indicate that in the alkaline situation a larger proportion of the body tissues with which the blood equilibrates at a high rate is included. The permeability increase is consistent with the physicochemical properties of the doxorubicin molecule and the changes in the calculated microconstants k_{21} and k_{12}, although the latter are not significant.

Previous investigators have found renal excretion varying from 1% of the administered dose after 8 h [2] to 3%–4% after 1 h [12], which is reasonably in keeping with the present findings. From Fig. 2 it appears that the doxorubicin clearance shows a pronounced decline over time. Initially the doxorubicin clearance was 20–25 ml/min, but it declined to approximately half this value at the end of the observation period. A corresponding fall in total clearance has been reported previously following IV administration of daunorubicin 10 mg/kg in dogs [15].

The declining doxorubicin clearance could result from a nephrotoxic effect due to initial high plasma concentrations of doxorubicin, but this explanation is not very likely because the inulin clearance is unchanged throughout the experiment.

The higher doxorubicin clearance in relation to inulin clearance must be caused by tubular mechanisms. The renal handling of doxorubicin can then be explained by (1) a secretion mechanism with high capacity and (2) a reabsorption mechanism with limited capacity (Fig. 3).

The reabsorption mechanism shows saturation at plasma concentrations above 0.14 μg/ml and 0.12 μg/ml for the acidic and alkaline states, respectively. Above these concentrations secretion is dominant, showing no sign of saturation within the concentrations measured in the experiment.

A similar finding has been made by Crom et al. [4] in an investigation of cisplatin. They found that the ratio of cisplatin clearance to creatinine clearance during the first 3 h, when the plasma concentrations are highest, is significantly higher than during the rest of the experimental period.

A mechanism like the one described by Christophidis et al. [3] for the renal handling of methotrexate in humans, where the changes in renal clearance are explained by changes in the synthesis rate of a carrier protein in the renal tubular cells, cannot be ruled out. The time needed for development of this effect is about 10–12 h, which is much longer than the duration of the present experiment, but the possibility of a doxorubicin-transporting carrier protein with a higher rate of turnover in the rabbit kidney cells could be considered.

The differences in renal clearance of doxorubicin between the acidic and alkaline states at the same plasma concentrations may be explained to some extent by the observed changes in inulin clearance. But even when a correction is made, the clearance of doxorubicin is higher in the alkaline state than in the acidic state at all plasma concentrations measured. This implies that the secretion mechanism functions more efficiently in the alkaline than in the acidic state.

This may be explained by suggesting that the secretion mechanism in the tubular cells is situated at or in the luminal membrane, and that the rate of transport is limited by the supply from the peritubular phase. In the alkaline state the pharmacokinetic calculations suggest an increased tissue permeability, which may then increase the supply of doxorubicin to the secreting mechanism.

A similar distinction between the processes by which a substance is transferred from the blood side into the tubular cells and from the cells into the lumen has been shown for p-aminohippurate [7].

From the renal clearance of doxorubicin (Fig. 3) it is further seen that there might be a difference in the maximal capacity of the reabsorbing mechanism, where the maximal rate in the alkaline is lower than in the acidic state estimated by the plasma concentration. This is consistent with the concept that the tubular reabsorption mechanism works on the charged species of the doxorubicin molecule. In the acidic urine doxorubicin will be more markedly dissociated than in the alkaline urine.

The increase in total body clearance, Cl_B, calculated as $k_e \cdot V_1$, cannot be explained by the increase in renal excretion alone. Cl_B is increased by approximately 100% from the acidic to the alkaline state, whereas the renal clearance is only increased by approximately 20%. This implies that the extrarenal clearance must also be increased.

The renal excretion of the major metabolite doxorubicinol is not significantly different in the two situations, implying that the biliary excretion must be increased in the alkaline state, which is consistent with an increased cellular permeability and thereby an increased supply to the biliary transporting mechanism.

In conclusion, the renal handling of doxorubicin may be described as (1) glomerular filtration, followed by (2) tubular secretion, and finally by (3) reabsorption, which is saturable within the range of the plasma concentrations found in the experiments.

The secretion rate is limited by the rate of access of doxorubicin from the blood side, and the reabsorption rate, by the amount of ionized doxorubicin in the urine, i.e., on the luminal side of the tubular cells.

The differences in doxorubicin pharmacokinetics between the acidic and alkaline states may have clinical/toxicological consequences in cancer patients with acid-base disturbances and should be further investigated.

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