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Short communication

Doxorubicin and doxorubicinol plasma concentrations and excretion in parotid saliva*

Françoise Bressolle[1], Jeanne-Marie Jacquet[2], Marc Galtier[3], Jacques Jourdan[2], Daniel Donadio[4], and Jean-François Rossi[5]

[1] Département de Pharmacocinétique, Faculté de Pharmacie, Montpellier, France
[2] Laboratoire Universitaire de Thérapeutique, Faculté de Médecine, Montpellier-Nimes, France
[3] Laboratoire de Pharmacocinétique, Pharmacie Carémeau, C. H. R. U. de NIMES, avenue du Pr. Debré, F-30006 Nimes Cedex, France
[4] Service des Maladies du sang, Hôpital Lapeyronie, Montpellier, France
[5] Institut du Cancer – Clinique Val d'Aurelle et INSERM U291, Montpellier, France

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Summary. The pharmacokinetics of doxorubicin (DOX) and doxorubicinol (DOXol) was studied in six patients with various advanced neoplastic diseases who received 28–72 mg/m^2 DOX (nine courses). Plasma and parotid saliva were collected over a 48-h period, and DOX and DOXol were quantified by high-performance liquid chromatography with fluorescence detection. As reported previously, a wide range of plasma levels were found among our patients. It appears that in addition to being quickly cleared from the plasma, both DOX and DOXol are excreted in detectable amounts in parotid saliva, a route of elimination that has been given little attention, if any. Excretion in the saliva exposes the mucosa of the upper gastrointestinal tract to drug and may play a role in causing stomatitis in patients receiving DOX by the i.v. route. Since huge interindividual and pronounced intraindividual differences were found in S/P ratios that mostly were not systematically related to the plasma drug concentration, the concentration in parotid saliva was not useful in predicting the level of free DOX and DOXol in plasma. For the parent drug and its metabolite, the S/P ratios increased significantly with time during the 48-h period after dosing.

Introduction

Doxorubicin (DOX) is one of the major antineoplasic agents used in the treatment of non-Hodgkin's lymphoma and diverse solid tumors [1, 6, 9–11, 13]. DOX is rapidly distributed throughout tissues and is slowly eliminated in bile [12]. Although the plasma concentration-time course and the excretion of DOX and its metabolites in bile and urine have been studied, little information is available on the detectability and excretion of these drugs in saliva [2, 3].

In the present study, the decline of DOX and its major metabolite doxorubicinol (DOXol) in plasma and their excretion in parotid saliva, a route of elimination heretofore given little attention, if any, were investigated. Ratios of parotid saliva to plasma (S/P) concentrations were calculated, and the importance of parotid salivary excretion as a route of elimination of these agents was evaluated.

Patients and methods

Patient population. A total of six patients were included in the study; two of them received two to three successive courses of chemotherapy. None of the subjects had previously been treated with radiotherapy regimens. All patients underwent a physical examination before their inclusion in the study. Their status was ascertained by standard laboratory tests, liver-function tests (bilirubin, prothrombin time, alanine aminotransferase, aspartate aminotransferase), and renal function tests as well as urinalysis and urine microscopy. Cardiac function (ECG, echocardiogram, and/or angioscintigraphy) was also checked. None of the patients showed signs of renal or hepatic dysfunction. Informed consent was obtained from each subject and for each treatment course. The patients' characteristics and the chemotherapy regimens involved are summarized in Table 1.

Drug administration and doses. A 10-min infusion of DOX was given by an infusion pump in a peripheral vein. The delivered dose varied from 28 to 72 mg/m^2 and was invariable for each patient throughout the treatment. Courses were repeated every 4 weeks. Administration of the other drugs was carried out at least 18 h after the DOX infusion.

Blood sampling. For each subject, baseline parotid saliva and blood samples were taken prior to the injection of drug for the calculation of calibration curves. Blood samples were collected from an indwelling venous catheter in ethylenediaminetetraacetic acid (EDTA)-coated tubes at the end of the infusion and at 10, 20, and 40 min and 1, 2, 4, 6, 12, 24, 36, and 48 h postinfusion. Parotid saliva samples were collected by means of a modified double-lumen parotid cup. Orange-flavored lozenges served as a reflex stimulus to induce salivation. At least 2 ml parotid saliva was collected over a period not exceeding 5 min; samples were collected at 2, 4, 6, 12, 24, 36, and 48 h postinfusion. Following the separation of plasma from blood, both plasma and parotid saliva samples

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Offprint requests to: F. Bressolle, Département de Pharmacocinétique, Faculté de Pharmacie, 11 Avenue Charles Flahault, F-34 060 Montpellier Cedex 01, France

Table 1. Patients' characteristics

Patient	Sex (M/F)	Age (years)	Diagnosis/histology	Treatment	Doxorubicin (mg)	Courses (*n*)
1	M	61	NHL-DSCC	m-BACOD	75	2
2	M	65	NHL-DLC	AVCB	130	3
3	F	51	Breast cancer	AVCF	60	1
4	F	63	NHL-DLC	m-BACOD	70	1
5	F	66	Breast cancer	AVCF	70	1
6	M	31	Hodgkin's disease	ABVD	45	1

NHL, Non-Hodgkin's lymphoma; DSCC, diffuse small-cell, cleaved; DLC, diffuse large-cell; m-BACOD, methotrexane/bleomycin/doxorubicin/cyclophosphamide/vincristine/dexamethasone; AVCB, doxorubicin/vindesine/cyclophosphamide/bleomycin/methylprednisolone; AVCF, doxorubicin/vindesine/cyclophosphamide/5-fluorouracil; ABVD, doxorubicin/bleomycin/vinblastine/dacarbazine

Table 2. Individual pharmacokinetic parameters determined for DOX

Patient	Course	Dose (mg/m^2)	Plasma						Parotid saliva			
			AUC[a] ($\mu g\ l^{-1}\ h$)	$t_{1/2}$ dis1 (h)	$t_{1/2}$ dis2 (h)	$t_{1/2}$ elim (h)	V_d (l)	C_T[b] (l/h)	AUC[a] ($\mu g\ l^{-1}\ h$)	$t_{1/2}$ elim (h)	t_{max} (h)	c_{max} (ng/ml)
1	1	44.12	58.3	0.0853	1.40	34.3	1,444.1	29.7	10.5	53.2	2.17	11.7
	2	44.12	58.9	0.0488	0.275	17.0	686.9	29.4	7.26	18.2	4.17	12.1
2	1	72.2	36.5	0.111	1.06	21.7	1,545.7	47.4	41.3	20.3	23.2	66.5
	2	72.2	25.4	0.0671	2.14	20.9	2,141.5	68.1	55.2	47.7	2.17	75.1
	3	72.2	36.6	0.0991	1.62	23.7	1,678.2	47.2	23.4[c]	Not measurable		
3	1	37.5	44.1	0.0724	2.11	51.8	2,709.2	39.2	43.7	20.0	6.17	49.3
4	1	42.4	57.3	0.0861	4.57	51.2	2,133.7	30.2	91.9	36.8	4.17	82.6
5	1	41.18	30.2	0.0489	0.267	11.1	903.6	57.2	17.6	6.1	4.17	47.3
6	1	28.1	69.2	0.0479	0.205	26.0	870.4	25.0	50.7	20.1	12.17	39.6

[a] Normalized for dose (1 mg/m^2)
[b] Expressed for 1.73 m^2 body area
[c] An increase in the DOX concentrations over 48 h was observed, the AUC was computed using a model-independent approach, and AUC_{0-48} was computed instead of $AUC_{0-\infty}$)

were frozen at –20° C, and plasma and parotid saliva analyses were performed during the subsequent 2 weeks.

Analytical method. Concentrations of DOX and DOXol were determined in all samples by high-pressure liquid chromatography (HPLC) with fluorescence detection [7, 8].

Pharmacokinetic analysis. The plasma concentrations of DOX and DOXol versus time followed tri- and bicompartmental exponential decay patterns, respectively. The coefficients and exponents of the exponential terms were estimated using the extended least-squares method for DOX and the weighted $[1/Y(calc)^2]$ least-squares method for DOXol [4, 5]. The model used to fit the variation in plasma DOXol concentration versus time takes into account a re-increase in DOXol plasma levels during the terminal phase [6]. The elimination half-life was determined from the slope of the log-linear part of the curves.

The area under the plasma concentration-time curve (AUC) was calculated using the equation $AUC = AUC_{0\to T} + AUC_{T\to th} + c_{th}/\alpha$ (T = infusion time); $AUC_{0\to T}$ was calculated by the integral of the fitted model between 0 and T; and $AUC_{T\to th}$ was calculated by the trapezoidal rule, with *cth* representing the concentration at the last sampling time and α representing the rate of the terminal log-linear phase. To compute the DOXol AUC in saliva, a model-independent approach was used, except for subject 1 during the first course, because of an increase in DOXol concentrations between 2 and 48 h, which prevented calculation of the c_{th}/α term; therefore AUC_{0-48} was computed instead of $AUC_{0-\infty}$. The total body clearance (C_T) was calculated from the ratio of the DOX dose to the AUC, and the volume of distribution in equilibrated tissues was calculated as:

$V_d = C_T/\alpha$.

Statistical analysis. Plasma concentrations were plotted against parotid saliva concentrations, and ratios of parotid saliva to plasma (S/P) concentrations were plotted against time. Linear regression was performed using unweighted least-squares analysis of the data. The significance of the regression was confirmed using the *F*-test.

Results

After the end of the infusion, the distribution of DOX to peripheral tissues was followed by an apparent biexponential decline in plasma concentration as a function of time. The plasma data were fitted to a three-compartment open model. The individual pharmacokinetic parameters determined for each patient are presented in Table 2.

In parotid saliva, DOX was detected at between 2 and 4 h after drug administration. The highest observed concentration (c_{max}) as normalized for a 1-mg/m^2 dose, 1.04 ± 0.567 ng/ml, was reached at 2–24 h after drug administration, depending on the patient. In four subjects (patients 1, 2, 3, and 6) at 4 h postdosing, the DOX concentrations in parotid saliva were lower than the corresponding plasma levels; the S/P ratios were 0.248 ± 0.109 at $t = 2$ h and 0.447 ± 0.235 at $t = 4$ h ($n = 7$), whereas in the other two subjects (patients 4 and 5), these ratios were higher than 1.4. With the exception of patient 1, at beyond

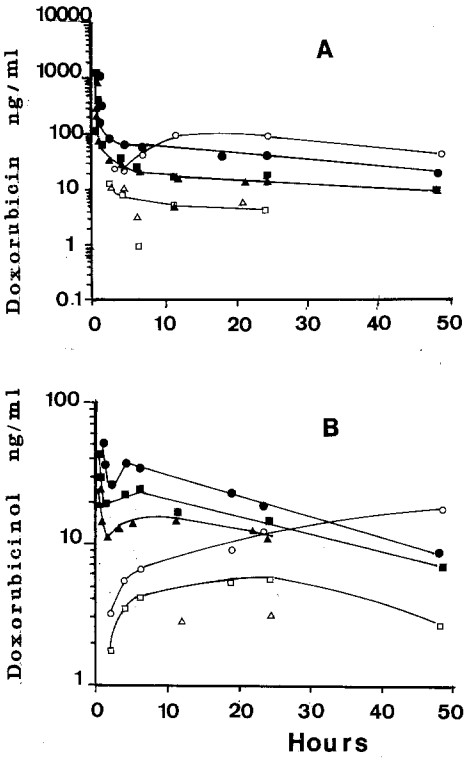

Fig. 1 A, B. Semilogarithmic plot of **A** DOX and **B** DOXol levels in plasma (*filled symbols*) and parotid saliva (*open symbols*) versus time following the administration of 75 mg DOX to patient 1 (■, □, first course; ▲, △, second course) and 130 mg DOX to patient 2 (●, ○, first course)

6 h after drug administration, the S/P ratios were greater than 1. Linear regression analysis revealed that DOX concentrations in parotid saliva and those in plasma were not correlated. When the S/P ratios were plotted against time, a statistically significant straight line could be fitted with a correlation coefficient of 0.504 (47 *df*, $P < 0.001$) and a slope of 0.0493 (Fig. 2A). After the highest observed concentration had been reached, the concentration of DOX in parotid saliva versus time followed a monoexponential decay pattern. In many cases, the elimination half-life was very near the value determined from plasma data (Table 2). Illustrations of the elimination of unchanged drug in plasma and parotid saliva are presented in Fig. 1 A. Patient 1 displayed very close elimination half-lives over the two successive courses.

The main metabolite detected was DOXol; although other metabolites were present, they occurred in very small amounts and could not be quantified. The plasma data for DOXol were consistent with an open two-compartment model. The individual pharmacokinetic parameters determined for each patient are shown in Table 3.

In parotid saliva, DOXol was detected later than DOX. At 24 h postdosing, the DOXol concentrations in parotid saliva were lower than the corresponding plasma levels. Linear regression analysis revealed that DOXol concentrations in parotid saliva and those in plasma were not correlated. When the S/P ratios were plotted against time, a statistically significant straight line could be fitted with a correlation coefficient of 0.774 ($P < 0.001$) and a slope of 0.0206 (Fig. 2B); the values ranged from a minimum of $0.155 \pm 5.69 \times 10^{-3}$ at $t = 4$ h to a maximum of 1.1 ± 0.60 at $t = 48$ h. For a great number of courses, the $t_{1/2}$ elimination value was not measurable; indeed, the DOXol concentration in parotid saliva increased during the 48-h period after dosing. Illustrations of the variations in DOXol concentrations in plasma and parotid saliva versus time are presented in Fig. 1 B.

Discussion

The plasma concentrations of DOX and DOXol dropped rapidly after the i.v. infusion, consistent with a previous report [6]. The large inter- and intraindividual variations in DOX and DOXol pharmacokinetic parameters are in agreement with the data reported in a previous study [6].

To date, few references have been made to the excretion of DOX and DOXol in parotid saliva. Only Celio et al. [3]

Table 3. Individual pharmacokinetic parameters determined for DOXol

Patient	Course	Dose (mg/m²)	Plasma			Parotid saliva	
			AUC[a] (μg l⁻¹ h)	$t_{1/2}$ elim (h)	R(AUC)[c]	AUC[a,b] (μg l⁻¹ h)	$t_{1/2}$ elim (h)
1	1	44.12	23.7	33.2	0.569	6.29	18.2
	2	44.12	26.7	50.3	0.854		Not interpretable
2	1	72.2	17.3	30.2	0.474	6.95	Not measurable
	2	72.2	12.6	39.2	0.496	1.46	Not measurable
	3	72.2	21.2	57.9	0.579	2.60	Not measurable
3	1	37.5	19.2	36.1	0.435	4.50	Not measurable
4	1	42.4	25.0	35.0	0.436	7.93	Not measurable
5	1	41.18	18.0	24.9	0.545	3.13	Not measurable
6	1	28.1	95.8	26.1	1.38	2.98	Not measurable

[a] Normalized for dose (1 mg/m²)

[b] When an increase in the DOXol concentrations over 48 h was observed (subjects 2–6), the AUC was computed using a model-independent approach, and AUC_{0-48} was computed instead of $AUC_{0-\infty}$. For subject 1, the data for the second course were insufficient

[c] Ratio of AUC DOXol/AUC DOX

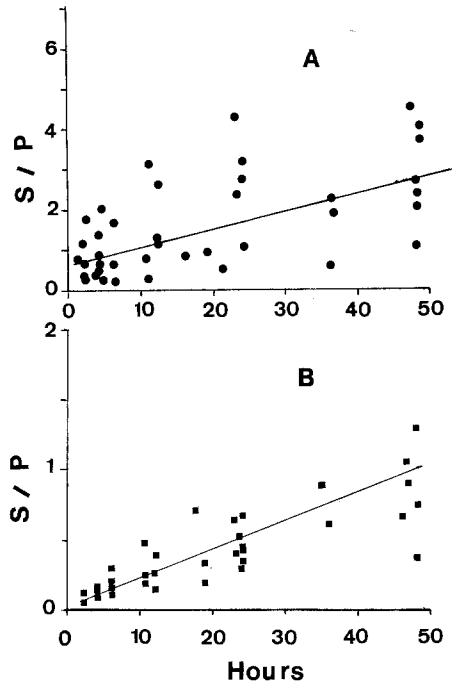

Fig. 2 A, B. Plot of **A** DOX and **B** DOXol S/P ratios versus time

have studied the excretion of DOX in parotid saliva over a 75-min period postdosing in eight patients who received 50–90 mg DOX by the i.v. route. The S/P ratios were 0.0925 ± 0.044, 0.21 ± 0.0676, and 0.23 ± 0.0312, at 15, 45, and 75 min after dosing, respectively. The present study demonstrates that both DOX and DOXol are excreted in detectable amounts in parotid saliva and that the S/P ratios increase significantly with time during the 48-h period after dosing. The plasma levels (P) represented the total fraction of the drug (free and bound to plasma proteins), taking into account a percentage of free DOX amounting to $15\% \pm 0.5\%$ to $20.6\% \pm 0.4\%$ for concentrations ranging from 60 to 1,900 ng/ml [3]. At 2 h postdosing, the concentrations of DOX in parotid saliva were higher than the corresponding concentrations of free DOX in plasma; at 6 h after dosing, the DOX concentrations in parotid saliva were about 5-fold the concentrations of free drug in plasma. The correlation between the DOX elimination half-lives in plasma and saliva seemed to be good except in two cases; thus, the salivary elimination half-life might be used to predict the plasma elimination half-life.

DOX and DOXol are excreted in noticeable amounts in parotid saliva, exposing the mucosa of the upper gastrointestinal tract to these agents even following i.v. administration of DOX; their excretion in the saliva may play a role in causing stomatitis in patients receiving DOX by the i.v. route. Since huge interindividual and pronounced intraindividual differences were found in S/P ratios, the concentration of drug in parotid saliva does not appear to be useful in indirect, noninvasive estimations of levels of unbound DOX in plasma.

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