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PHARMACOKINETICS OF DOXORUBICIN IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS AFTER INFUSION OF DOXORUBICIN-LOADED ERYTHROCYTES

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1. ABSTRACT

Pharmacokinetics of doxorubicin infused as a solution or doxorubicin-loaded erythrocytes was studied in three patients with lymphoproliferative disorders. Homologous or autologous erythrocytes were loaded with doxorubicin aseptically by their incubation at 37°C in the solution of doxorubicin for 1 h. During the i.v. infusion of doxorubicin in solution (25–50 mg per square meter of body surface area), its concentration in blood rose to 3.60–5.90 ug/ml. Doxorubicin declined to about 0.1 ug/ml 20–30 min postinfusion and became zero 12–24 hours later. When these patients received the same doses of doxorubicin loaded into erythrocytes, doxorubicin in blood did not exceed 2.5 ug/ml. Within 10–30 min after the end of the infusion, doxorubicin decreased to about 0.1 ug/ml and remained at this level for 3 days and longer. Administrations of doxorubicin-loaded erythrocytes produced no adverse side effects.

2. INTRODUCTION

Anthracycline antibiotics are potent anticancer agents that are broadly used for treatment of various malignant diseases. However, their high toxicity may cause severe adverse effects[7,14,16,18]. The use of erythrocytes as carriers of anthracycline antibiotics is promising for decreasing antibiotic toxicity and increasing its specific activity. Under certain conditions, intact erythrocytes can bind considerable amounts of anthracycline antibiotics[2,4,5,19,20]. However, this binding is weak and the antibiotics easily leave the erythrocytes[3,8,11,19,21]. It is possible to immobilize anthracycline antibiotics within erythrocytes by glutaraldehyde[3,9,19,21]. The use of

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doxorubicin-loaded erythrocytes (DLE) increases the lifetime of the antibiotic in the circulation and can provide target delivery of this antibiotic to spleen, liver, and lungs decreasing the antibiotic burden for other organs[10,15,21]. DLE were superior to the standard doxorubicin preparation in suppressing liver metastases in mice[22]. A clinical attempt to treat massive liver metastases with DLE was reported[20]. DLE were also used to treat lymphosarcoma in dogs[15]. However, along with high anticancer activity, DLE displayed pronounced myelotoxicity that caused severe pancytopenia in dogs[15]. In the cited studies, doxorubicin was immobilized in erythrocytes with glutaraldehyde. Glutaraldehyde treatment is likely to modify doxorubicin and this modification is associated with an uncontrollable change in the antibiotic toxicity. However, the efficacy of erythrocytes loaded with anthracycline antibiotics seems to be independent of how tightly antibiotics are bound within cells. Daunorubicin-loaded erythrocytes prepared without glutaraldehyde treatment were shown to display higher anticancer activity[11,12] and lower toxicity[1] than daunorubicin in solution. Therefore, it is a plausible suggestion that erythrocytes loaded with doxorubicin without glutaraldehyde treatment will also be superior to doxorubicin in solution.

This study is the first step of the clinical trial of DLE prepared without glutaraldehyde treatment and is aimed at evaluating their pharmacokinetics and tolerance.

3. MATERIALS AND METHODS

Three patients with lymphoproliferative disorders were studied. All they received chemotherapy according to the protocol involving doxorubicin treatment. Patient characteristics are briefly summarized in Table 1.

We used adriablastin produced by Farmitalia Carlo Erba (Montedison Group, Italy) for infusion as solution (standard form) and for preparation of DLE. To prepare DLE we dissolved the desired amount of adriablastin in physiological saline (70 ml) and introduced it into the blood bag containing packed erythrocytes (200 ml) preheated to 37°C. Erythrocytes were either homologous (for patient A) or autologous (patients B and C). After being thoroughly mixed, cells were incubated at 37°C for 1 h. These temperature and time of incubation provided almost complete binding of the antibiotic[4,20]. At the end of incubation, saline (100 ml) was added into the bag. All the manipulations were performed under sterile conditions. The suspension of DLE was infused to the patient immediately after preparation.

We studied the pharmacokinetics of doxorubicin in each patient on two separate occasions. In the first study, the patient received a single standard dose of doxorubicin in so-

Table 1. Characteristics of patients

Patient	A	B	C
Sex	F	M	M
Age	63	21	16
Diagnosis	Lymph.[a]	Lymphog [b]	Lymphog [b]
Disease stage	IV	IVBb	IVBb
Chemotherapy protocol including doxorubicin	CHOP	ABVD	ABVD
The number of chemotherapy cycles preceding the infusion of DLE	3	3	1
Doxorubicin per infusion (mg/m^2)	50	25	25

[a]Lymph. is the abreviation of Lymphosarcoma
[b]Lymphog. is the abreviation of Lymphogranulomatosis

lution. In the second study, this patient received the same dose of doxorubicin through the infusion of DLE. Patient A was treated with doxorubicin in solution and DLE within the two consecutive cycles of CHOP. Patients B and C received infusions of both types within one cycle of ABVD. Standard doxorubicin in solution and suspension of DLE were infused into the cubital vein for 10 min and 1–3 h, respectively. The first sample of blood for doxorubicin determination was drawn immediately after the end of the infusion. Further blood sampling was performed at various time intervals within 3 days postinfusion. Blood-preserving solution Glugicir, an analog of ACD, was used for anticoagulation. Each blood sample was split into two portions. One portion was immediately frozen. The second portion was centrifuged, and the supernatant plasma was separated from cells and frozen. Frozen samples of blood and plasma were stored at -20°C.

Blood and plasma samples were thawed, and 1.6 M K_2CO_3 (0.1 ml) and chloroform (3 ml) was added to 1 ml of the sample. The mixture was vigorously stirred and centrifuged at 1000 g for 3 min. The chloroform extract of doxorubicin at the bottom of the tube was carefully collected with a glass syringe. Doxorubicin in the chloroform extracts was determined spectrofluorometrically (476-nm excitation and 588-nm emission). Solutions of doxorubicin in chloroform of the known concentrations were used for calibration. The lower detection limit of the method was approximately 10 ng/ml blood or plasma.

4. RESULTS

Despite significant individual differences, the pharmacokinetics of doxorubicin in different patients displayed several general features in common (Fig. 1). Immediately after the end of the infusion of the doxorubicin solution, its concentration in the blood was 3.6–5.9 ug/ml. Doxorubicin sharply declined within 30 min postinfusion by 30-fold or greater and remained at the level of approximately 0.1 ug/ml for several hours. The concentration of doxorubicin fell to zero 12–24 hours postinfusion. When the same doses of doxorubicin were infused loaded in erythrocytes, the pharmacokinetics changed dramatically (Fig. 1). Immediately after the end of the infusion, blood doxorubicin was significantly lower (1.44–2.5 ug/ml) than that after infusion of doxorubicin solution. Within 10–30 min after the end of the infusion of DLE, blood doxorubicin also sharply decreased; however, it remained at the level of 0.1 ug/ml throughout the observation period being nonzero even at 72 h postinfusion. Blood and plasma pharmacokinetics of doxorubicin did not differ.

The different pharmacokinetics of doxorubicin delivered as solution or DLE resulted in the significant differences in the areas under the pharmacokinetic curves (Table 2). In the case of DLE, the 72-h area is several times larger than that observed after the infusion of doxorubicin solution. Patients well tolerated DLE. No immediate or postinfusion adverse reactions were noticed.

5. DISCUSSION

The pharmacokinetic curves for doxorubicin solution observed in this study are similar to those reported by others[6,13,20]. The use of DLE significantly changed the pharmacokinetics of blood doxorubicin. In our opinion, the main difference is a manyfold increase in the circulation time of the antibiotic. Similarly increased circulation times of doxorubicin were observed in dogs that received erythrocytes loaded with doxorubicin and treated with glutaraldehyde[10,15]. In these experiments, blood doxorubicin remained at a level lower than

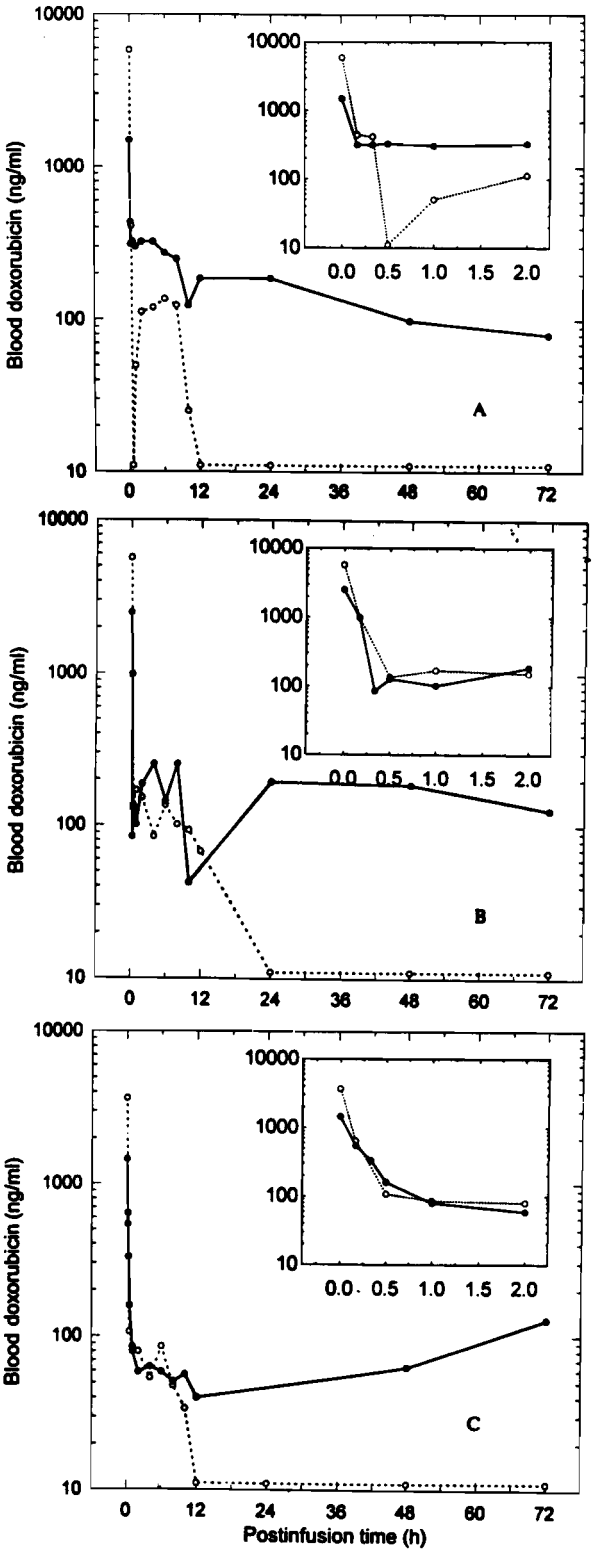

Figure 1. Individual kinetics of blood doxorubicin after infusion of its solution (open circles) or doxorubicin-loaded erythrocytes (dark circles) in (A) patient A, (B) patient B, and (C) patient C. Portions of curves showing the first 2 h postinfusion are in separate frames.

Table 2. The area under the pharmacokinetic curve as a function of time elapsed from the infusion of doxorubicin solution or doxorubicin-loaded erythrocytes (DLE). The areas were calculated from data presented in Fig. 1

	Area under curve (ug h/ml)					
	Patient A		Patient B		Patient C	
Postinfusion time (h:min)	Doxorubicin solution	DLE	Doxorubicin solution	DLE	Doxorubicin solution	DLE
00:00	0.0	0.0	0.0	0.0	0.0	0.0
00:10	0.524	0.150	0.555	0.288	0.357	0.165
00:20	0.595	0.202	-	0.377	-	0.238
00:30	0.628	0.255	0.742	0.395	0.482	0.278
01:00	0.642	0.412	0.818	0.452	0.530	0.338
02:00	0.722	0.678	0.978	0.597	0.613	0.408
04:00	0.953	1.24	1.22	1.04	0.747	0.530
06:00	1.21	1.83	1.44	1.44	0.887	0.653
08:00	1.47	2.37	1.67	1.83	1.02	0.763
10:00	1.62	2.73	1.87	2.13	1.10	0.872
12:00	1.65	3.05	2.03	-	1.14	0.968
24:00	1.65	5.28	2.44	3.79	1.14	-
48:00	1.65	8.70	2.44	8.36	1.14	2.84
72:00	1.65	10.85	2.44	12.12	1.14	5.16

the level that we observed in our patients. It should be noted, however, that we determined doxorubicin from the total fluorescence of samples, and it remained uncertain whether only doxorubicin or doxorubicin and products of its metabolism were present in the samples. Nevertheless, our results suggest that the use of DLE prepared without the tight immobilization of the antibiotic within the cell by glutaraldehyde is highly promising. The technique for preparing such erythrocytes is very simple. Their use allows doxorubicin to circulate significantly longer and thereby may significantly enhance its therapeutic efficacy. In addition, the loaded erythrocytes prepared without glutaraldehyde treatment are better tolerated than those treated with glutaraldehyde. Administration of glutaraldehyde-treated erythrocytes is sometimes associated with adverse side effects[17,20].

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