Doxorubicin pharmacokinetics in lymphoma patients treated with doxorubicin-loaded eythrocytes

Doxorubicin-loaded erythrocytes (DLE) were administrated to 15 lymphoma patients. Antibiotic peak concentration in blood decreased by 55%, doxorubicin circulated several times longer, and the area under the concentration-time curve increased 5 times if compared with standard doxorubicin administration. The DLE was well tolerated by patients.

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Anthracycline antibiotics, including doxorubicin, are among the most utilized anticancer agents and are highly toxic. It is a general view that the use of various vehicles to carry drugs in the organism is a promising way to enhance drugs efficacy while reducing their toxicity. The use of erythrocytes for drug delivery is an extremely fascinating perspective.[1,2] The technique of erythrocyte loading with antracycline antibiotics is very simple and efficient because erythrocytes bind these antibiotics in isotonic media.[3,4] Several cases of the use of erythrocytes loaded with anthracycline antibiotics in veterinary and clinical practice have been reported.[4-7]

In the present study we investigated the possibility of preparing doxorubicin-loaded erythrocytes (DLE) using patient blood. Pharmacokinetics of doxorubicin in fifteen patients with lymphomas was investigated after administration of DLE prepared using autologous patient blood (AB-DLE) and erythrocytes (AE-DLE) or donor erythrocytes (DE-DLE). Doxorubicin binding to erythrocytes in citrated patient whole blood *in vitro* was studied as described elsewhere.[3,4] Patients with 3rd-4th stage lymphomas, relapsed, with bad tolerability or resistant to standard chemotherapy, were included in the pharmacokinetics study (Table 1). Statistical analysis included data, obtained earlier in three patients treated with DLE.[7] The experimental protocol was approved by the Scientific Council of the National Research Center for Hematology. Methods and risks were explained and written consent was obtained from each patient. Doxorubicin was administered as a part of the CHOP or ABVD protocol of chemotherapy. Standard doxorubicin was administered intravenously in 50 ml of 0.9% NaCl. To prepare DLE, doxorubicin solution in 0.9% NaCl was added to a plastic blood pack containing 100-200 mL of preserved patient blood, patient erythrocytes or donor erythrocytes to obtain a final dose of 25 or 50 mg per m^2 after infusion. The mixture was incubated at 37°C with constant agitation for one hour. DLE was infused to patients immediately after preparation. Blood samples for pharmacokinetics analysis were collected, and doxorubicin concentration was measured as described elsewhere.[3,4,7] The first sample was drawn immediately after completing drug infusion. The subsequent samples were each drawn every 5-10 min during the 30 minutes following drug infusion, and then at increasing intervals from 30 minutes to 8-10 h within 24 h (totally 8-10 samples). Then samples were drawn daily for the subsequent 5-7 days, until doxorubicin concentration dropped to its quantitation threshold of ~10 ng/mL. The time dependences of blood and plasma doxorubicin concentration in the pharmacokinetic studies were approximated by a sum of two exponentials:

$$C=Cb+A1exp(-t/T1)+A2exp(-t/T2)$$

Here, C is doxorubicin concentration at time *t* after the end of infusion; Cb is doxorubicin quantitation threshold; A1 and A2 are coefficients; T1 and T2 are the time con-

Table 1. Patient characteristics at the begining of the study.

Patient Code	Sex	Age (years)	Hb (g/L)	WBC (10^9/L)	Crea (μmol/L)	Bilir (mmol/L)	Diagnosis	Treatment
Kol[a]	F	63	–	–	–	–	Aggressive NHL, relapsed	Free(50)1 + DE-DLE(50)1
Gra[a]	M	21	–	–	–	–	Hodgkin's lymphoma, 4B, relapsed	Free(25)1 + AE-DLE(25)1
Sto[a]	M	16	–	–	–	–	Hodgkin's lymphoma, 4B, relapsed	Free(25)1 + AE-DLE(25)1
Kuz	M	40	144	6.3	90	12	Aggressive NHL, 3B, relapsed	Free(50)1
Dgu	M	18	-	-	-	-	Hodgkin's lymphoma, MC, 4B, relapsed	Free(25)1
Wis	M	35	106	5.6	80	18	Hodgkin's lymphoma, NS, 4B, relapsed	Free(25)1 + DE-DLE(25)1
Ger	M	20	86	3.6	63	7	Aggressive NHL, 4B, relapsed	Free(25)1 + DE-DLE(25)1
Ost	M	20	99	2.9	70	4	Hodgkin's Lymphoma, 4B, relapsed	Free(25)1 + DE-DLE(25)1
Jom	F	75	92	7.0	95	5	Diffuse B-large cell NHL, relapsed	Free 50(1) + AB-DLE(50)2 +DE-DLE(50)1
Abd	M	32	118	76.0	100	12	Diffuse B-large cell NHL, relapsed	AB-DLE(50)1
Kak	M	80	130	4	90	15	Diffuse B-large cell NHL, relapsed	AB-DLE(25)1 + DE-DLE(25)1
Kal	M	60	–	–	–	–	Diffuse B-large cell, NHL, relapsed	AB-DLE(25)2
Ser	F	55	112	4.0	70	6	Centrofollicular lymphoma in transformation,relapsed	AB-DLE(50)1 + AE-DLE(50)1
Kur	F	73	145	9.0	75	9	Diffuse B-large cell NHL, relapsed	AB-DLE(50)1 + AE-DLE(50)4
Mat	F	52	138	12.1	74	15	Diffuse B-large cell NHL, relapsed	AE-DLE(50)2
Fro	F	73	117	4.9	60	11	Aggressive NHL, relapsed	AE-DLE(50)5
Gri	M	75	109	17.9	90	6	Mantle cell lymphoma in transformation	AE-DLE(50)2
Sad	F	37	–	–	–	–	Hodgkin's lymphoma, NS, 4B, relapsed	DE-DLE(50)1
Che	M	46	–	–	–	–	Hodgkin's lymphoma, 4B, relapsed	DE-DLE(25)1
Bon	M	43	107	2.5	81	7	Hodgkin's lymphoma, NS, 4B, relapsed	DE-DLE(50)1

Hb, WBC, Crea, and Bili: mean hemoglobin, white blood cells, creatinine, and bilirubin respectively; NHL: non- Hodgkin's lymphoma, not immunophenotyped, NS: nodular sclerosis of Hodgkin's lymphoma. MC: mixed cellularity Hodgkin's lymphoma. The "Treatment" column shows a sequence of doxorubicin infusions for each patient in the following format: Doxorubicin form(dose in mg/m^2)number of infusions. [a]Initial data for this patient were taken from Ataullakhanov FI, Isaev VG, Kohno AV, Kulikova EV, Parovichnikova EN, Savchenko VG, et al. Pharmacokinetics of doxorubicin in patients with lymphoproliferative disorders after infusion of doxorubicin-loaded erythrocytes. In: Sprandel U, Way JL, editors. Erythrocytes as Drug Carriers in Medicine. New York, London: Plenum Press; 1997. p 137-42.

Table 2. The average values of specific times of doxorubicin clearance in fast (T1) and slow (T2) phases obtained in blood and plasma of patients after infusion of doxorubicin solution (Free) and doxorubicin-loaded erythrocytes (AB-DLE, AE-DLE, and DE-DLE).

Doxorubicin form	*Free*	*AB-DLE*	*Blood AE-DLE*	*DE-DLE*	*Free*	*Plasma AB-DLE*	*AE-DLE*	*DE-DLE*
Number of patients	9	6	7	9	9	6	7	9
Number of infusions	9	8	16	9	9	8	16	9
T1, (h)	**0.16±0.06**	**0.29±0.05**	**0.49±0.13**	**1.07±0.35**	**0.1±0.0**	**0.36±0.07**	**0.58±0.19**	**0.69±0.13**
Significance of the difference between free drug and DLE administration, (p)		<0.05	<0.04	<0.02		<0.002	<0.05	<0.0002
T2, (h)	**7.3±2.0**	**15.8±6.6**	**28.6±6.8**	**48.4±8.8**	**5.5±1.3**	**16.8±7.3**	**19.4±3.9**	**39.9±11.2**
Significance of the difference between free drug and DLE administration, (p)		<0.11	<0.02	<0.0002		<0.08	<0.02	<0.04

Data are presented as mean±SE. Statistical significance was analyzed using two-sample t-test.

stants of the first and the second exponentials respectively (specific times of doxorubicin clearance). The values of T1 and T2 were determined using the Microcal Origin 3.5 software (Microcal Software, Inc.).

Data are presented as mean±SE. We have found, that in patient blood, *in vitro* erythrocytes bind doxorubicin with the equilibrium erythrocyte/plasma concentration ratio of 2.8±0.3 (n=6) achieved in 30-60 min. While this ratio is significantly lower than the ratio of 4-5 obtained in suspension of washed erythrocytes,[3] the doxorubicin in patient blood is mainly bound by erythrocytes. Thus, the patient blood can be used for preparation of DLE.

Two phases, fast and slow, were distinguished in doxorubicin clearance from blood and plasma independent whether the antibiotic was infused in the free or the DLE form, as earlier observed.[7,8] The drug concentration dropped rapidly during the first 10-30 min after the infusion decreasing by one or two orders of magnitude. It then declined slowly to the lower limit of its quantitation. In six patients treated with equal doses of standard and DLE doxorubicin forms the average blood and plasma antibiotic peak concentrations after DLE infusion were respectively 45±5% and 34±11% of the values observed after infusion of standard doxorubicin form. In only one patient doxorubicin peak concentrations were higher after DLE infusion. The specific times of fast and slow phases of doxorubicin clearance increased several times in case of DLE administration, compared with standard doxorubicin form (Table 2). Comparison of the areas under the concentration-time curve in patients receiving the same dose of doxorubicin as standard form and as DLE shows that after DLE infusion the area was on average 4.8±1.4 times higher for blood (n=9) and 6.4±1.5 times higher for plasma (n=8).

The DLE was well tolerated by patients. No prolonged or severe myelosuppression was observed. No evidence of cardiotoxicity was seen. Moreover, DLE was infused without any negative consequences to a patient (Ser) who earlier responded to standard doxorubicin form with strong paroxismal tachycardia and cardialgia, and to another patient (Jom) presenting ciliary arrhythmia.

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