Comparison of Efficacy, Toxicity and Pharmacokinetics of Free Adriamycin and Adriamycin Linked to Oxidized Dextran in Rats

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Adriamycin linked to oxidized dextran (ADM-OXD) *via* Schiff's base formation was compared with free adriamycin with regard to antitumor activity, acute toxicity and plasma pharmacokinetics in rats following i.v. administration. ADM-OXD showed higher activity against Walker carcinosarcoma 256 than free adriamycin. On the other hand, the acute toxicity of ADM-OXD was adout three times less than that of free adriamycin. In contrast to free adriamycin, a very high plasma level of adriamycin was found after i.v. administration of ADM-OXD. The area under the plasma concentration curve with ADM-OXD was about 160-fold higher than with free adriamycin. Thus, the improvement of the therapeutic index in the case of ADM-OXD might be due to the difference in the disposition of ADM-OXD and free adriamycin in rats.

Keywords adriamycin; high molecular weight prodrug; dextran; *in vivo* antitumor activity; pharmacokinetics; acute toxicity

Anthracycline antibiotics, adriamycin and daunomycin, are among the most important and useful drugs in cancer chemotherapy. However, the doses of these drugs are severely limited because of their cardiotoxicity.[1] In an attempt to improve the therapeutic index of the drugs, conjugates with macromolecules such as dextran,[2] poly-L amino acid,[3] and deoxyribonucleic acid (DNA)[4] have been investigated, as well as chemical derivatives or conjugates with tumor specific antibodies. Pratesi *et al.*[5] reported that the therapeutic index of adriamycin was improved by conjugation with poly L-aspartic acid in mice. Levi-Shaffer *et al.*[6] found that the toxicity of daunomycin in mice was reduced by conjugation with dextran.

In this investigation, we prepared adriamycin-oxidized dextran conjugate (ADM-OXD) and compared the therapeutic index (the ratio of toxic dose to effective dose) of ADM-OXD with that of free adriamycin in rats. Furthermore, the relationship between the therapeutic index and plasma pharmacokinetics of the two drugs was discussed.

Experimental

Preparation of ADM-OXD Adriamycin was purchased from Kyowa Hakko Kogyo Co. (Tokyo, Japan). Dextran T-70 was purchased from Pharmacia Japan. (Tokyo, Japan). Dextran T-70 (1.0 g) was dissolved in 10 ml of distilled water and incubated with 30 ml of 0.41 M $NaIO_4$ for 5 h at room temperature in the dark to oxidize some of the glucose residues. After dialysis against an excess volume of 50 mM phosphate buffer (pH 7.5), 700 mg of glycine was added to the oxidized dextran solution and the Schiff's base formed between glycine and the oxidized dextran was reduced with $NaBH_4$ to stabilize the bond. Glucose residues remaining in the resulting dextran-glycine were fully oxidized by addition of $NaIO_4$ (2.5 g) to the solution. The oxidized dextran-glycine was obtained by dialysis against distilled water and lyophilization. Then adriamycin (10 mg) and the oxidized dextran-glycine (40 mg) were reacted in 10 ml of 50 mM phosphate buffer (pH 7.5) at room temperature for 3 h. The resulting ADM-OXD was precipitated and purified by addition of 4 volumes of acetonitrile. Adriamycin content in ADM-OXD (7—10%) was determined by measuring the absorbance at 495 nm.

Testing of Antitumor Activities The experiments with Walker carcinosarcoma 256 were carried out in Wistar rats inoculated i.m. with 1×10^6 cells per rat. ADM-OXD and free adriamycin were dissolved in physiological saline and administered once i.v. 5 d after the inoculation of the tumor. Nine days later, the tumor weight of each treatment group was compared with that of the untreated group. Growth inhibition rate was calculated as follows;

$$\text{growth inhibition rate } (\%) = (1 - T/C) \times 100$$

where T and C are the tumor weight of the treatment group and that of the untreated group, respectively.

Toxicity Test ADM-OXD and free adriamycin were administered i.v. to normal male rats at various doses and LD_{50} was calculated from the mortality during 28 d.

High Performance Liquid Chromatography (HPLC) The HPLC apparatus used consisted of a Rheodyne model 7125RV injector, Shimadzu model LC-4A pump, and Hitachi model 650-10S fluorescence detector set at 470 nm for excitation and 580 nm for emission. HPLC analysis was performed using a Cosmosil $5C_{18}$ packed column (150 × 4.6 mm i.d., Nacalai Tesque Inc., Kyoto, Japan) with a mobile phase of 30% acetonitrile in 1% triethylamine formic acid buffer (pH 4.0) at a flow rate of 1.5 ml/min.

Determination of Adriamycin in Blood Plasma Blood of rats was collected from polyethylene tubing cannulated into the carotid 4—6 times during 360 min after i.v. administration of ADM-OXD or free adriamycin at a dose of 10 mg/kg. The dose of ADM-OXD is expressed as adriamycin content in this paper. Plasma was separated by centrifugation at 2500 rpm for 20 min. Free adriamycin concentration was determined by HPLC as follows: blood plasma was extracted with 10 volumes of $CHCl_3$–MeOH (4 : 1) mixture and the extract was dried under nitrogen gas. The residue was dissolved in 0.1 ml of the mobile phase for HPLC and subjected to HPLC analysis. Adriamycin concentration was determined spectrophotometrically when ADM-OXD was administered. Plasma samples were diluted 8 times with 50 mM phosphate buffer and the concentration of total adriamycin was determined by measuring the absorbance at 495 nm.

Pharmacokinetic Analysis The plasma concentration *versus* time data after i.v. administration at the dose of 10 mg/kg were analyzed with the two compartment model.

Results

Antitumor activities of ADM-OXD and free adriamycin against Walker carcinosarcoma 256 were tested with a single i.v. administration 5 d after the tumor inoculation (Table I). At a dose of 0.67 mg/kg, the inhibition rate by ADM-OXD was about 50%, while that by free adriamycin was only about 20%. When the dose was increased to 2.0 mg/kg, the inhibition rates by ADM-OXD and free adriamycin were about 81% and 63%, respectively. Thus, ADM-OXD had higher antitumor activity than free adriamycin against this solid tumor.

In order to estimate the improvement of therapeutic index, the lethal toxicity of ADM-OXD in normal rats was compared with that of free adriamycin. Mortality of rats after single i.v. administration of ADM-OXD or free adriamycin is shown in Table II. The toxicity of adriamycin was markedly reduced when it was linked to oxidized dextran and the LD_{50} values of free adriamycin and ADM-OXD were calculated to be 10.4 mg/kg and more than 25.8 mg/kg, respectively. The results on efficacy and toxicity

TABLE I. Effects of ADM-OXD and Free Adriamycin on Walker Carcinosarcoma 256

	Dose (mg/kg)[a]	Experiment 1 Tumor weight (g)	Experiment 1 Inhibition rate (%)	Experiment 2 Tumor weight (g)	Experiment 2 Inhibition rate (%)
No treatment		6.02±2.48[b]	—	6.76±3.16	—
ADM-OXD	0.67	2.91±2.11	51.7	3.48±2.18	48.5
	2.0	0.90±0.88	85.1	1.54±1.21	77.3
Free adriamycin	0.67	N.D.[c]		5.32±1.90	21.2
	2.0	1.98±1.29	67.1	2.79±1.80	58.7

The drugs were given i.v. 5 d after i.m. inoculation of Walker carcinosarcoma 256. The tumor weight was measured 9 d after the treatment. *a*) In the case of ADM-OXD, the dose is expressed as adriamycin content. *b*) Mean±S.D. of ten rats per group. *c*) Not done.

TABLE II. Comparative Toxicity of ADM-OXD and Free Adriamycin on Single i.v. Administration in Rats

Drug	Dose[a] (mg/kg)	No. of rats treated	No. of survivors[b]	LD_{50}[a] (mg/kg)
ADM-OXD	12.4	10	10	
	14.9	10	10	
	17.9	10	10	More than 25.8
	21.5	10	10	
	25.8	10	9	
Free adriamycin	6.0	10	10	
	7.2	10	9	
	8.6	10	9	10.4
	10.4	10	6	
	12.4	10	0	

a) In the case of ADM-OXD, the dose and LD_{50} are expressed as adriamycin content. *b*) Survivors on the 28th day.

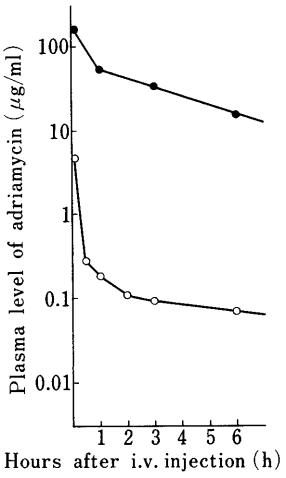

Fig. 1. Plasma Levels of Adriamycin after i.v. Administration of ADM-OXD and Free Adriamycin at a Dose of 10 mg/kg as Adriamycin

●, ADM-OXD; ○, free adriamycin. Each point represents the mean of triplicate samples.

indicated that the therapeutic index of ADM-OXD was markedly improved as compared with that of free adriamycin.

Plasma level of adriamycin was measured after i.v. administration of ADM-OXD or free adriamycin at a dose of 10 mg/kg (Fig. 1). The plasma level of adriamycin decreased rapidly after administration of free adriamycin. The plasma level of the drug was 4.67 μg/ml at 5 min after the administration and decreased to 0.18 μg/ml during 1 h. On the other hand, the plasma level of adriamycin 5 min after the i.v. administration of ADM-OXD was 163.7 μg/ml, and gradually decreased to 54.4 μg/ml during 1 h. Comparative pharmacokinetic parameters are shown in Table III. The volume of distribution of the central compartment (V_c) with free adriamycin was about 30 times larger than that with ADM-OXD. Clearance of ADM-OXD from rat plasma was also very slow as compared with that of adriamycin. The areas under the plasma concentration curve (AUC) with ADM-OXD and free adriamycin were estimated to be 302.1 and 1.9 μg·h/ml, respectively.

TABLE III. Plasma Pharmacokinetics of Free Adriamycin and ADM-OXD

	$AUC_{0\to\infty}$ (μg h/ml)	V_c (ml/kg)	V_p (ml/kg)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (h)	Clearance (ml/h/kg)
Free adriamycin	1.91	1016.3	9201.5	4.5	2.9	5236.7
ADM-OXD	302.1	33.78	89.82	3.9	4.4	33.10

Discussion

The present study demonstrated that adriamycin linked to oxidized dextran (ADM-OXD) had not only higher antitumor activity but also less toxicity than free adriamycin, as shown in Tables I and II. Levi-Schaffer *et al.*[6] reported that the therapeutic index of daunorubicin was improved when it was linked to oxidized dextran. However, the antitumor effect of the conjugate was much less than that of free daunorubicin at the same dose. The discrepancy between our results and the results reported by Levi-Schaffer *et al.* might be due to whether the Schiff's base formed between the drug and oxidized dextran was reduced or not. Namely, the Schiff's base in daunorubicin-oxidized dextran prepared by Levi-Shaffer *et al.* was reduced by sodium borohydride but ADM-OXD used in this study was prepared without reduction of the Schiff's base between the drug and oxidized dextran. Antitumor activity of these macromolecular prodrugs is considered to be manifested following release of the drug from the macromolecule, because the cytotoxicity of adriamycin or daunorubicin requires intercalation into DNA.[7] Therefore, the reduction of the Schiff's base to strengthen the bond between the drug and macromolecules was disadvantageous.

The plasma pharmacokinetics of adriamycin after i.v. administration of ADM-OXD was very different from that after i.v. administration of free adriamycin. The area under the plasma concentration curve of the drug after injection of ADM-OXD was 160-fold higher than that with free adriamycin. Antitumor activity is not always predictable

from the plasma concentration of an antitumor drug, but a change of pharmacokinetics could improve the efficacy and toxicity of a conventional antitumor drug. Macromolecules are easily taken up by tumor tissues because tumor neovasculature has a leaky character, different from the vascular systems in normal tissues.[8)]

The markedly reduced toxicity and increased efficacy of ADM-OXD may be related to the high plasma level of the drug and the elevated permeability of the tumor neovasculature. Another explanation for the improvement of the therapeutic index is that conjugation of adriamycin to oxidized dextran may alter the metabolism and excretion of adriamycin. The mechanism of antitumor effect of ADM-OXD, including the metabolism, is under investigation. In summary, the significant alteration of the therapeutic index and pharmacokinetics of adriamycin when linked to oxidized dextran suggest potential for the improvement of clinical cancer chemotherapy.

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