Cardiotoxicity of Anthracycline Antineoplastic Drugs

— Clinicopathological and Experimental Studies —

TADASHI SUZUKI, HIROSHI KANDA, YASUHIRO KAWAI, KEIGO TOMINAGA,*
AND KAZUHIKO MURATA

THE myocardial damage is not infrequently associated with the administration of drugs and chemicals in man and in experimental animals.¹ Adriamycin (ADR) and Daunomycin (DNR), two antineoplastic drugs of the anthracycline type, have a high degree of oncolytic activity not only against acute leukemia but also against solid tumors. However, the prolonged use of these drugs have been limited by the development of the heart failure.²⁻⁷ It is well established that the cardiotoxic effects are dose-dependent and the previous reports indicate that a total cumulative dose less than 500 mg/m² or 550 mg/m² yields a very low incidence of cardiomyopathy, whereas the incidence of cardiomyopathy climbs rapidly in patients receiving a greater total dose.^{4,8} On the other hand, the exact pathogenetic mechanism of the delayed chronic cardiotoxicity of anthracyclines is still unknown, although several complex biochemical alterations have been reported to be produced by ADR and DNR.⁹ Olson¹⁰ and Jaenke¹¹ reported an increase in tissue calcium in the hearts of rabbits treated with ADR and they proposed calcium-mediated necrosis as one important mechanism.

In the first part of the present paper, a clinical and pathological studies were made in order to determine the incidence of cardiotoxicity of an-

thracycline antineoplastic drugs and to find out characteristic morphological changes of cardiac cells produced by these drugs. In the second part, experimental studies were carried out in order to elucidate the pathogenesis of anthracycline induced myocardial lesions.

I. Clinical and pathological studies

Materials and methods

The studies were made on 162 cases of hematopoietic neoplasm treated from 1970 to 1978. Of these, 110 cases, 56 males and 54 females, were administered ADR and/or DNR, while the remaining 52 cases, 25 males and 27 females, were not treated with anthracyclines. The latter cases consisted of control group. The ages of the patients ranged from 8 to 79 years in the anthracycline group, while from 11 to 79 years in the control group. The anthracycline treatment was performed as conventional way combined with concurrent antineoplastic therapy including vincristine, cytosine arabinoside, 6-MP, prednisolone and cyclophosphamide. The total cumulative dose ranged from 25 to 747 mg/m² with a mean dose of 290 mg/m². The follow up period was from 1 to 59 months in the anthracycline group and from 1 to 68 months in the control group.

An autopsy was performed in 61 of 110 anthracycline treated cases and 28 of 52 control cases. Routine light microscopic examinations of the hearts were made on all autopsy cases, while electron microscopic studies of the hearts were made on 9 anthracycline treated cases according to the routine method in our laboratory.

Key Words:

Adriamycin
Daunomycin
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The second Department of Internal Medicine, Gunma University School of Medicine

* *National Cancer Center Hospital*

TABLE I ECG ABNORMALITIES

	Total	Treated with anthracycline		Control
		Dose < 400 mg/m ²	Dose ≥ 400 mg/m ²	
No. of cases	110	77	33	52
No. of cases with ECG abnormalities	41	24	17*	15
ST-T change	34	19	15	8
arrhythmia				
(tachyarrhythmia, premature contraction, ...)	33	19	14	7
others (low voltage, LAD, ...)	3	3	0	2

* $P < 0.05$

Fig.1. Myofiber degeneration with multiple intracellular vacuolization and interstitial fibrosis. Autopsy case treated with anthracycline. H-E stain; $\times 435$.

Fig.2. Intracellular vacuoles resulting from dilation of sarcoplasmic reticulum. Autopsy case treated with anthracycline. $\times 6700$.

Results

Electrocardiographic abnormalities, such as sinus tachycardia, premature beats and ST-T changes, were demonstrated in 41 of 110 (37.3%) cases treated with anthracycline and in 15 of 52 (28.8%) control cases. The electrocardiographic abnormalities were significantly more frequently observed in the cases received large dose of an-

thracycline more than 400 mg/m² (17 of 33 cases, 51.5%) than in the remaining cases ($P < 0.05$) (Table I).

Although the congestive heart failure was complicated in 4 (3.6%) of anthracycline treated cases with cumulative dose from 409 to 675 mg/m², this was not a direct cause of death in any case. The congestive heart failure occurred

Fig. 3. The incidence and the severity of the morphological cardiotoxicity in relation to anthracycline total dose.

from 5 to 11 months after the start of the therapy and from 2 to 6 weeks after the last administration.

In autopsy cases, mild leukemic infiltrates and small bleeding lesions of the hearts were frequently demonstrated. Cloudy swelling, vacuolar degeneration or basophilic degeneration of the cardiac cells, interstitial edema and focal hypertrophy of left ventricular muscle cells were also frequently observed both in anthracycline group and in control group. However, a characteristic vacuolar degeneration of the cardiac muscle cells were observed only in 16 cases received anthracycline. The myofiber alterations appeared to the progressive formation of multiple intracellular dilatations, leading occasionally to extensive vacuolization in which myocardial cells assumed an appearance of worm-eaten spots (Fig. 1.). The eventual myolysis of myocardial cells and inter-

stitial fibrosis were observed in association with an extensive degeneration. The electrocardiographic abnormalities were demonstrated in 10 of 16 (62.5%) cases with characteristic myocardial lesions.

In the myocytes with the above-mentioned characteristic vacuolar degeneration, multiple intracellular vacuoles resulting from dilatation of sarcotubular system were demonstrated (Fig. 2.). The finding was obvious in 16 cases received a total dose from 221 to 675 mg/m². The incidence and the severity of the myocardial lesions advanced as the total cumulative dose of anthracycline was increased (Fig. 3.).

Among the cases received total dose of 200 mg/m² or greater, the characteristic myocardial lesions were more frequently observed in the cases with abnormal electrocardiogram before treatment and in those with significant coronary

TABLE II MORPHOLOGICAL CARDIOTOXICITY IN PATIENTS RECEIVED TOTAL DOSE OF 200 mg/m² OR GREATER

<i>Pre-treatment ECG</i>	<i>No. of patients</i>	<i>Age (yrs) mean ± SE</i>	<i>Dose (mg/m²) mean ± SE</i>	<i>No. of patients with morphological cardiotoxicity</i>
<i>normal</i>	24	38.1 ± 3.5	374.3 ± 32.2	8 (33.3%)
<i>abnormal</i>	12	42.1 ± 4.1	495.1 ± 34.3	8 (66.7%)
<i>Coronary sclerosis</i>	<i>No. of patients</i>	<i>Age (yrs) mean ± SE</i>	<i>Dose (mg/m²) mean ± SE</i>	<i>No. of patients with morphological cardiotoxicity</i>
(+)	11	53.0 ± 4.3	382.6 ± 39.4	7 (63.6%)
(-)	24	33.0 ± 2.7	433.2 ± 34.0	9 (37.5%)

sclerosis at autopsy (Table II.). The characteristic myocardial lesions were demonstrated in 8 of 12 (66.7%) cases with abnormal electrocardiogram prior to the treatment and 7 of 11 (63.6%)

cases with significant coronary sclerosis at autopsy, while in 8 of 24 (33.3%) cases with normal electrocardiogram before the treatment and 9 of 24 (37.5%) cases without significant coronary

sclerosis.

Comment

It is generally accepted that the chronic cardiotoxic effects of anthracycline are related to the cumulative dose administered. The present study revealed a significantly high incidence of electrocardiographic changes in the cases received large dose of anthracycline of more than 400 mg/m². The characteristic multiple intracellular vacuolization was also more frequently observed in those treated with large doses of anthracycline, while all the cases complicated with congestive heart failure received more than 400 mg/m².

Although myocardial lesions of various types were observed both in anthracycline and control groups, characteristic multiple intracellular vacuoles resulting from the dilatation of sarcotubular system were demonstrated only in the anthracycline group. Similar findings were reported in biopsy and autopsy cases received anthracycline treatment^{3,5,6,12} as well as in the experimental anthracycline toxicity^{10,13}

Although the cardiotoxicity of anthracycline is dose-related, other additional factors may be contributory. The characteristic myocardial lesions were more frequent in those with significant coronary sclerosis and in those with abnormal electrocardiograms prior to the treatment.

II. Experimental studies

Materials and methods

ADR was administered to 45 male white rabbits weighing from 1.8 to 2.2 kg via the marginal ear vein at a dose of 2.5 mg/kg (27.5 mg/m²) once a week. ADR was reconstituted in isotonic saline at a concentration of 2.0 mg/ml. Seven rabbits died 8 weeks after the start of the ADR administration or later. Other rabbits were sacrificed 1, 2, 3, 5, 8 and 10 weeks after the initial administration. Under nembutal anesthesia, the blood samples were collected for the determinations of hemoglobin, leukocytes count, serum electrolytes concentrations and enzyme activities. Light microscopic and electron microscopic studies of the heart were performed according to the conventional method. A part of left ventricular tissue, about 1 g in wet weight, was dried in an oven, ashed with sulfonitric acid, and the tissue electrolyte contents were determined with atomic absorption spectrophotometry thereafter.

In addition to the above experiments, the protective effect of verapamil, a calcium antagonist,

against ADR toxicity was studied. One mg/kg verapamil was given subcutaneously every 12 hours, in addition to the weekly intravenous administration of ADR. The rabbits were sacrificed 5, 8 and 10 weeks after the initial injection.

Results

As shown in Fig. 4, myocardial lesions were frequently observed with the increase of cumulative dose of ADR. Seven of 22 cases received more than 220 mg/m² died before sacrifice, and 5 of these showed overt evidences of congestive heart failure such as ascites, hydrothorax, hydropericardium and subcutaneous edema.

Light microscopic examinations revealed scattered myocardial degeneration and necrosis even at small total dose of 138 mg/m². The lesions were more frequent and extensive, and associated with interstitial fibrosis in the cases received more than 220 mg/m². The lesions were characterized by multiple vacuolization of myocardial cells, interstitial edema and fibrosis, and disorganization and disappearance of the cross-striated pattern of myofibrils (Fig. 5). Some myocardial cells were occupied by many vacuoles of various sizes.

The ultrastructural examination showed the progressive dilatation of the sarcoplasmic reticulum and a part of transverse tubular system, leading occasionally to extensive vacuolization and ballooning of myocytes (Fig. 6). Accompanied with the sarcotubular dilatation, myofibrils frequently formed contraction band and their parallel orientation was lost. The myofibrils occasionally showed progressive disruption with lysis of myofilaments (Fig. 7). An almost complete disappearance of myofibrils was observed in some myocytes. In these myocytes, this finding was not necessarily accompanied with morphological changes of mitochondria and sarcoplasmic reticulum. The mitochondria showed various stages of degeneration. In some areas with marked myofibrillar disorganization, a few mitochondria contained amorphous dense bodies or ring-shaped inclusions composed of small dense particles in the matrix (Fig. 8). At later stages, sarcoplasm of some myocardial cells was occupied by a large amount of degenerating mitochondria and laminated myelin figures, which appeared to have been derived from sarcoplasmic reticulum (Fig. 9). The nuclear changes were not remarkable in general.

The most outstanding change of myocardial electrolyte contents was the increase in the tis-

Fig. 7. Disappearance and decrease of myofibrils. Apparent mitochondrial degeneration is absent in this area. ADR-treated rabbit, 275 mg/m². $\times 5200$.

Fig. 8. Ring-shaped inclusions ($\downarrow$) in some mitochondria of a severely degenerated myocardial cell. These inclusions are composed of small dense particles. ADR-treated rabbit, 275 mg/m². $\times 30000$.

Fig. 9. Multilaminated myelin figures in a degenerated myocardial cell. ADR-treated rabbit, 275 mg/m². $\times 10000$.

TABLE III MORPHOLOGICAL ALTERATIONS, ELECTROLYTE AND WATER CONTENTS OF MYOCARDIUM (MEAN $\pm$ SE)

morphological alterations histological score	myocardial electrolytes (mmoles/100g)								myocardial water content (%)
	calcium		magnesium		sodium		potassium		
	wet	dry	wet	dry	wet	dry	wet	dry	
(-)	0.10 ±0.00	0.50 ±0.02	0.87** ±0.03	4.20*** ±0.10	3.96 ±0.20	19.29 ±1.05	7.38 ±0.36	35.57 ±1.28	79.4 ± 0.4
(+)	0.10 ±0.00	0.46 ±0.01	0.77 ±0.05	3.68 ±0.29	4.16 ±0.09	19.83 ±0.08	7.88 ±0.23	37.55 ±0.35	79.0 ± 0.2
(++)	0.13* ±0.01	0.63* ±0.05	0.87*** ±0.02	4.28*** ±0.09	4.24 ±0.23	20.85 ±1.25	7.78 ±0.44	38.21 ±2.23	79.2 ± 0.3
(+++)	0.16** ±0.01	0.81* ±0.09	0.75 ±0.04	3.84 ±0.12	4.84 ±0.38	25.42 ±3.10	8.00 ±0.16	41.57 ±2.06	80.6 ± 0.9
controls	0.10 ±0.00	0.48 ±0.02	0.76 ±0.01	3.61 ±0.08	3.88 ±0.15	18.46 ±0.77	7.37 ±0.28	35.00 ±1.26	78.8 ± 0.4

* $p < 0.05$ ** $p < 0.02$ *** $p < 0.01$ TABLE IV MORPHOLOGICAL ALTERATIONS, ELECTROLYTE AND WATER CONTENT OF MYOCARDIUM (MEAN $\pm$ SE)

myocardial morphological alterations				myocardial electrolytes (mmoles/100g)								myocardial water content (%)
histological score	vacuolization of myocytes (%)	interstitial tissue area (%)	calcium		magnesium		sodium		potassium			
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry		
ADR (220–275mg/m ²) n = 5	3.2 ±0.4	5.7 ±1.4	26.7 ±3.7	0.14 ±0.00	0.69 ±0.04	0.82 ±0.04	4.18 ±0.19	4.81 ±0.26	24.81 ±2.05	7.79 ±0.63	39.71 ±0.55	79.95 ±0.78
ADR (138mg/m ²) n = 3	1.0 ±0.0	0.7 ±0.3	19.8 ±2.1	0.10 ±0.00	0.47 ±0.01	0.79 ±0.06	3.69 ±0.30	3.95 ±0.05	18.56 ±0.47	8.03 ±0.07	37.70 ±0.62	78.70 ±0.29
ADR (220–275mg/m ²) + verapamil, 1 mg/kg s.c.q 12h n = 4	3.5 ±0.3	2.5 ±0.7	21.9 ±1.8	0.14 ±0.00	0.68 ±0.03	0.89 ±0.01	4.23 ±0.10	5.07 ±0.09	23.94 ±0.40	8.02 ±0.17	37.89 ±0.63	78.82 ±0.44
ADR (138mg/m ²) + verapamil, 1 mg/kg s.c.q 12h n = 3	1.3 ±0.3	1.4 ±0.6	18.8 ±2.0	0.10 ±0.00	0.47 ±0.02	0.88 ±0.01	4.15 ±0.11	3.90 ±0.10	18.37 ±0.47	8.16 ±0.31	38.47 ±0.60	78.80 ±0.37

sue calcium in the cases with moderate to severe myocardial lesions (Table III). Slight and insignificant increase in tissue sodium concentration was also observed. The serum calcium was significantly low in the cases with moderate to severe myocardial lesions. The serum LDH, CPK and urea nitrogen levels were obviously elevated.

The verapamil treatment did not significantly affect the cardiotoxicity of ADR (Table IV). The light and electron microscopic alterations in verapamil treated rabbits were similar to those administered ADR alone. In spite of verapamil treatment, the ADR affected cell count, interstitial tissue area and the myocardial electrolyte changes were not reduced.

Comment

The present experimental studies demonstrated the dose-related cardiotoxicity of ADR. As pointed out by previous investigators,¹⁰⁻¹³ the characteristic morphological features of the myocardial lesions were (1) multiple intracellular vacuolization resulting from dilatation of sarcotubular system, (2) disorganization and disappearance of the myofibrils accompanied by lysis of myofilaments and (3) various mitochondrial degenerations and formation of myelin figures. Of these findings, the multiple intracellular vacuolization in the myocardial cells was the most unique alteration. The dilatation of the sarcotubular system was so marked that a similar degree of the change has never been observed in other types of cardiomyopathy, and suggest the intracellular water and electrolyte redistributions.¹⁴

Recent investigations have suggested that the altered electrolyte metabolism might significantly contribute the development of myocardial lesions.¹⁴⁻¹⁷ Increase in myocardial calcium in ADR-treated rabbits with marked myocardial degeneration was demonstrated in the present study. It may be conceivable that the ADR-induced myocardial lesions might result secondarily from the alterations in myocardial electrolyte metabolism, especially of calcium. However, verapamil, a calcium antagonist, could not protect the development of myocardial lesions. On the other hand, it has been pointed out that the ADR promote lipid peroxidation by means of reactions mediated by free radicals and injure the membrane system.¹⁸ Therefore, it may be also conceivable that the extensive cytoplasmic vacuolization and the alteration

of the myocardial calcium content are attributable to the disturbance of selective permeability of membrane system.

Almost complete disappearance of myofibrils was observed in some myocardial cells, and it is noteworthy to point out that this finding was not necessarily accompanied with morphological changes of mitochondria and sarcoplasmic reticulum. The selective myofibrillar degeneration raises a possibility that the inhibition of protein synthesis by ADR may cause the derangement of myofibrils.

III. Concluding remarks

A dose-related cardiotoxicity of ADR was demonstrated by present clinical, pathological and experimental studies. Among various morphological changes of the myocardial cells, the multiple intracellular vacuolization resulting from the dilatation of the sarcotubular system was the most striking finding. The calcium content of the heart was significantly increased in experimental ADR cardiomyopathy. It was postulated that the ADR-induced myocardial lesions may result secondarily from the extensive cytoplasmic vacuolization and the alteration of the cardiac calcium metabolism as a result of the disturbance of selective permeability of membrane system. On the other hand, based on the occasional observation of selective myofibrillar degeneration, a possibility was also considered that the inhibition of protein synthesis by ADR may cause the derangement of myofibrils.

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