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Adriamycin-induced Decrease of Myocardial Contraction Reserve in Rats Tested with Dobutamine*

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Abstract—*Muscle-mechanical and hemodynamic properties were measured in adriamycin-treated rats and in untreated controls. The respective parameters in both animal groups could not be distinguished at rest. Using a pharmacological stress test with dobutamine a differentiation of both groups was possible: the concentration–response curve of papillary muscles from ADM-rats was lower and shifted towards higher concentrations when compared with the respective curve of untreated animals. The maximum hemodynamic response following an i.v. bolus injection of dobutamine was considerably decreased in ADM-rats as compared to untreated rats. It is concluded that the estimation of impaired myocardial function due to adriamycin might be more accurate when the contraction reserve is evaluated.*

INTRODUCTION

THE IMPORTANCE of 'late cardiotoxicity' as a limiting factor in the antitumor therapy with adriamycin (ADM) has often been discussed [1–5]. The clinical course and pathological changes in man and experimental animals are well-characterized [6–9]. Biochemical and biophysical results from several experimental animal models refer to the possible modes of action of ADM which might be important in the development of the ADM-cardiomyopathy [10–14]. The registration of ADM-induced physiological changes in chronic experimental animal models are, however, not as well defined [15].

This study was therefore carried out to quantify the chronic effect of adriamycin on the myocardium of rats by measurements of physiological parameters of the myocardial function at rest and during pharmacological stimulation with dobutamine.

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Abbreviations used—ADM: adriamycin; b.w.: body weight; i.p.: intraperitoneally.

MATERIALS AND METHODS

Animals and pretreatment

Forty-day-old (131 ± 15 g) and 80-day-old (288 ± 31 g; $\bar{x} \pm$ S.D.) male Sprague–Dawley rats were pretreated with adriamycin doses of 1 mg/kg b.w./day i.p. on 3 consecutive days, with a subsequent rest of 4 days before the cyclic treatment was started again. Forty 'adriamycin-rats' and 40 untreated controls (NaCl i.p.) had free food (Altromin 1324) and water supply. Adriamycin was used as Adriblastin® (Farmitalia, Milan, Italy and Freiburg, F.R.G.).

Muscle-mechanical studies

For evaluation of representative muscle-mechanical properties we studied the isometric contractions of electrically stimulated papillary muscles from 14 pretreated rats after cyclic treatment with a final dose of 12–18 mg/kg b.w. and 17 untreated controls. Papillary muscles of less than 1 mm in diameter were excised from the right or left ventricle, incubated in 35°C Krebs–Ringer solution, gassed with 95% O_2 and 5% CO_2 ($pO_2 > 600$ mmHg) and stimulated by square wave impulses of 2.5 msec duration and a voltage of 50% above threshold at a stimulation frequency of 1 Hz by using the following

pulse generators: Typ E 161, Tektronik, Portland, U.S.A. and HG 100 F, Hivotonic Ltd., England. The developed tension (T) of the muscle under optimal resting tension and its first derivation (dT/dt) were recorded via a semiconductor transducer (AE 803, Aksjeselskapet Mikro-Elektronik, Horton, Norway) on a Gould multichannel recorder (Typ 2400, Gould Instruments, Cleveland, Ohio, U.S.A.) amplified by medium gain DC-amplifier (Mod. 13461510).

When steady state was reached, cumulative concentration–response curves for dobutamine (Dobutrex®, Lilly, Bad Homburg, F.R.G.) were determined by a logarithmic increase of dobutamine concentration in the incubation medium. Subsequent doses were administered immediately after the maximum effect of the preceding dose had been achieved.

The dobutamine-induced increase of maximum tension development (T_{max}) and maximum rise of tension (dT/dt_{max}) of pretreated rats and untreated controls was plotted as a percentage of pre-drug steady state values over the logarithmic concentration of dobutamine.

Hemodynamic studies

For the evaluation of representative hemodynamic parameters *in situ*, the following surgical procedure was done in 9 cyclic pretreated rats (total dose 12–18 mg ADM/kg b.w.) and in 7 untreated controls: the rats were thoracotomized in barbital or ether anesthesia following tracheotomy for respiration and insertion of venous and arterial catheters. The left ventricle was tapped with a steel cannula and connected to Statham transducers. An electromagnetic flowmeter of appropriate size (Hellige, Freiburg, F.R.G.) was placed around the ascending aorta. When needed, the heart was paced via the ventricular steel cannula. Using a Hellige multichannel recorder, pump, pressure and contractility parameters were continuously monitored. In this series of experiments we registered left ventricular pressure (P_{LV}), left ventricular pressure rise (dP_{LV}/dt), left ventricular end diastolic pressure (LVEDP), aortic pressure (P_A) and central venous pressure (CVP). Furthermore, ECG, heart rate, phasic and mean aortic flow (which is cardiac output (C.O.) minus coronary blood flow) were continuously recorded.

When steady state was reached, 50 μg dobutamine/kg b.w. was injected as an i.v. bolus. This dose had been identified as the maximally effective dose in preceding experiments. The resultant changes of the most important hemodynamic parameters were plotted as percentages of the respective initial values.

RESULTS

Response parameters of drug application

Figure 1 shows the effect of adriamycin on the body weight of 20 Sprague–Dawley rats, aged 40 days (Fig. 1A), and 20 rats, age 80 days (Fig. 1B), as compared to 20 respective control rats. After 4–6 weeks of pretreatment with adriamycin, body weight was about 30% lower in the ADM-treated group (Fig. 1).

The heart weight was 0.79 ± 0.1 and 0.81 ± 0.09 g in untreated animals and 0.64 ± 0.07 and

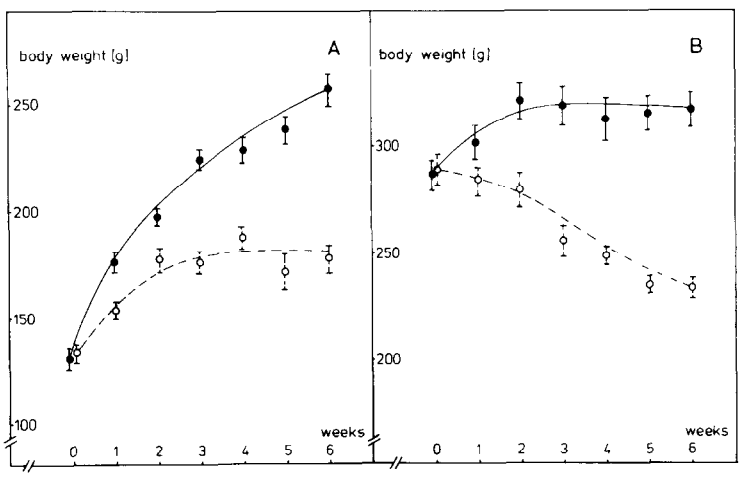

Fig. 1. Effect of adriamycin on body weight of growing rats (A) and adult rats (B). Twenty young male Sprague–Dawley rats and 20 adult rats were injected intraperitoneally (1 mg adriamycin/kg body weight on 3 consecutive days with a subsequent rest of 4 days). Each time point represents mean ± S.E.M. of 16–20 untreated rats (—●—) versus 16–20 rats pretreated as indicated by the time point (—○—).

0.69 ± 0.09 g in the pretreated groups, corresponding to 13–15% difference in pretreated versus untreated animals ($x \pm$ S.D.).

At a total dose of 12–18 mg/kg adriamycin, there was a significant effect on body weight, but only a slight effect on heart weight and no or minimal histological changes in the myocardium. The clinical condition of the rats in this dose range was good, except for diarrhea in several cases. No signs of heart failure could be seen in any of the rats.

Muscle-mechanical changes

The maximum isometric tension development of 17 papillary muscles of untreated rats was 0.815 ± 0.431 g/mm^2, and the corresponding value of 14 papillary muscles of ADM-treated animals (12–18 mg ADM/kg b.w.) was 0.706 ± 0.482 g/mm^2. Thus, control animals and chronically pretreated animals could not be distinguished by the absolute tension development.

Figure 2 demonstrates that untreated and pretreated rats could clearly be differentiated by dobutamine concentration–response curves. Whereas maximum tension development of papillary muscles from untreated rats could be increased by $96.3 \pm 11.4\%$ of initial values following dobutamine stimulation, the corresponding increase for papillary muscles of pretreated rats was only $57.8 \pm 23.2\%$ (Fig. 2A). The difference was even more pronounced for the maximum rise of tension development (Fig. 2B), where dobutamine-induced increase was $153.5 \pm 18.9\%$ for control rats as compared to $72 \pm 28.4\%$ for the ADM-treated group. Both dose–response curves were shifted to the right.

Hemodynamic results

As Fig. 3 shows, there was no significant difference in left ventricular pressure- and pump-parameters of untreated versus ADM-treated rats. Only the heart rate was significantly lower in pretreated animals. Parameters of contractility did not allow a differentiation between the two animal groups.

Figure 4 shows that untreated and ADM-treated rats can be easily differentiated by maximum dobutamine stimulation (i.v. bolus injection of 50 μg dobutamine/kg b.w.). In 7 control rats dobutamine caused an increase of heart rate, maximum left ventricular pressure, maximum left ventricular pressure rise and cardiac output by $41 \pm 14.5\%$, $75.1 \pm 27.9\%$,

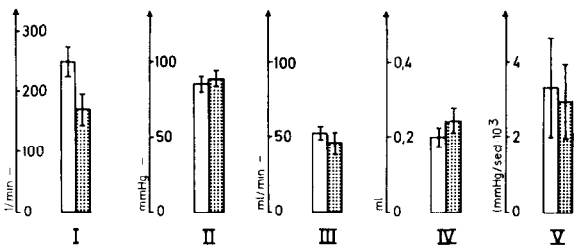

Fig. 3. Hemodynamic parameters of untreated open-chest rats (open bars) and pretreated open-chest (stippler bars) at rest. Adriamycin-rats had a cyclic pretreatment with 12–18 mg *adriamycin/kg b.w. (see methods). Untreated and pretreated rats were thoracotomized after anesthesia and tracheotomy. Pressure values were measured via a ventricular steel cannula, flow values were recorded via an aortic electromagnetic flowmeter. Each open bar represents mean ± S.D. of the indicated hemodyanmic parameters of 7 untreated rats, each stippled bar represents the respective values of 9 chronically pretreated rats. I = heart rate, II = ventricular pressure, III = cardiac output, IV = stroke volume, V = maximum rise of left ventricular pressure.*

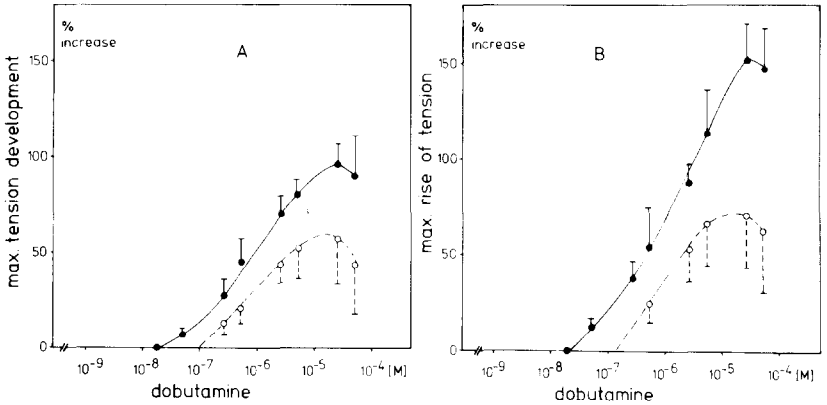

Fig. 2. Cumulative concentration–response curves for maximum isometric tension development (A) and maximum rate of tension development (B) in adriamycin pretreated rats (—○—) and untreated controls (—●—). Adriamycin-rats had a cyclic pretreatment with 12–18 mg *ADM/kg b.w. as described in Methods. The isometric contractions of isolated papillary muscles from pretreated rats and untreated controls were measured. After steady state, dobutamine was cumulatively added to the incubation medium and the resulting increase in the muscle-mechanical parameters was measured as percentage of steady state values. Each concentration point represents mean ± S.D. of the muscle mechanical response from* 14 *pretreated rats (—○—) and* 17 *controls (—●—).*

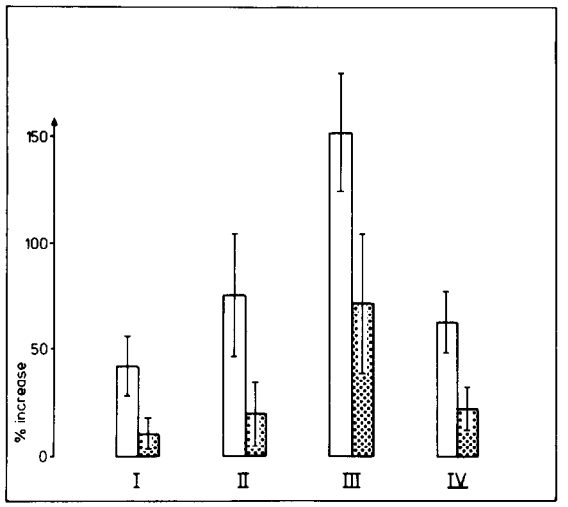

Fig. 4. Hemodynamic parameters of untreated open-chest rats (open bars) and pretreated open-chest rats (stippled bars) after maximum dobutamine stimulation. Adriamycin-pretreated rats (see Methods) and untreated controls were instrumented as described in Methods and briefly mentioned in Fig. 3. The hemodynamic response following an i.v. bolus injection of 50 μg dobutamine/kg b.w. was measured as the percentage change of steady state values. Each open bar represents mean ± S.D. of the indicated hemodynamic parameters of 7 untreated rats, each stippled bar represents the respective values of 9 chronically pretreated rats. I = heart rate, II = left ventricular pressure, III = maximum rise of left ventricular pressure, IV = cardiac output.

152 ± 29.2% and 62.2 ± 15% of initial values. In 9 ADM-treated rats the corresponding increase was much less, with 10.2 ± 5.9%, 19.5 ± 14%, 71.5 ± 32.1% and 22 ± 9.8% respectively.

Muscle-mechanical and hemodynamic results below and above 12–18 mg/kg *ADM*

Rats receiving less than 4 cycles of adriamycin pretreatment (9 mg/kg adriamycin or less) showed no reproducible changes in muscle-mechanical and hemodynamic properties, not even under conditions of maximal pharmacological stimulation.

Rats receiving more than 6 cycles of pretreatment (over 18 mg/kg adriamycin) showed an exponential rise of morbidity. Symptoms consisted primarily of severe diarrhea, signs of infections, leucopenia, anemia and thrombopenia. In gross pathological investigations of 27 rats no pleural effusions as a clinical sign of heart failure were seen. In several rats which had a total dose of more than 30 mg/kg adriamycin hemodynamic investigations were performed, but no changes of resting parameters could be seen when compared with controls.

DISCUSSION

The use of adriamycin as one of the most potent antitumor drugs is limited by a dose-dependent, severe, often lethal cardiomyopathy with sudden onset [2, 3, 4, 16]. The histopathological changes are well documented [6–9]. Various biochemical and biophysical investigations have been done to elucidate the pathogenesis of the adriamycin-cardiotoxicity [10–14, 17–20], and several agents have been suggested to protect the myocardium from the toxic effects of ADM or to counteract the adriamycin-cardiotoxicity [21–24].

However, there are no functional data from a respective chronic animal model which would allow correlation of biochemical findings with physiological data. So far, the effects of recommended cardioprotective substances have not been evaluated by muscle-mechanical and hemodynamic data.

In this communication we describe experiments which quantify the ADM-induced changes in myocardial function in rats using physiological parameters, integrating the finding of others [25–27] that pharmacological tests could extend the assessment of impaired myocardial function. Our data describe a sensitive animal model for the evaluation of physiological parameters of the myocardial function as changed by chronic administration of adriamycin. This model could be used as functional reference for biochemical investigations or for the examination of a variety of 'cardioprotective' agents.

The incidence of irreversible congestive heart failure after adriamycin therapy escalates with the increase of the total dose of drug [3, 4]; therefore, a dose-limit of 550 mg/m^2 has been recommended [1]. There is, however, a wide range in the myocardial tolerance of individual patients treated with ADM [2, 4]. Thus, clinical tests for early detection of adriamycin cardiomyopathy were developed to achieve an individual adaption of the maximum prospective dose [28, 29]. Many of these tests failed to recognize functional myocardial disorders at a time when morphological changes could already be demonstrated in myocardial biopsies [30]. Though a slight decrease in myocardial function does not necessitate a cessation of the drug [31, 32], finding a more accurate method of defining the myocardial function remains a major goal in monitoring adriamycin therapy.

In the experiments described in this paper we investigate whether pharmacological tests which stress the myocardial contraction reserve are more sensitive and exact in detection of ADM-induced changes in myocardial function than measurements at rest.

Our data demonstrate that in adriamycin-

pretreated rats physiological parameters of myocardial function at rest are not different from the corresponding parameters of untreated animals. There is, however, a clear discrimination between both groups when the contraction reserve is estimated by pharmacological stimulation.

Most likely, it is unimportant whether dobutamine, other suitable substances (for example, angiotensine) or exercise testing are used to test myocardial contraction reserve, as long as the test is well standardized.

Our data show that the evaluation of myocardial function under pharmacological stress condition (here: dobutamine) is more sensitive in detecting impaired myocardial function due to adriamycin than evaluation of myocardial function at rest. Whether this will allow an earlier detection of adriamycin cardiotoxicity in clinical conditions remains to be established.

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