Daunomycin-Induced Cardiomyopathy in Rats and Its Possible Therapy with Peptide Preparation Isolated from the Heart

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A well-known consequence of treating malignant diseases with anthracycline antibiotics (AA), and the first to be noted by clinicians, is their cardiotropy, resulting in cardiomyopathy, in some cases with progressive heart failure and even a lethal outcome. Due to the high affinity of AA to membranes and their ability to intercalate between DNA bases, the immediate and delayed effects of anthracycline therapy are manifested in inhibition of nucleic acid and protein synthesis, reduced activity of the mitochondrial respiratory enzymes, and disturbances of ion homeostasis. This results in an energy deficit, an overload with metabolically inactive calcium, and LPO activation in the cells, as well as impaired contractile function, and enhanced diastolic rigidity of the myocardium [5,7]. Bearing in mind that there are no effective means for protecting the myocardium under conditions of anthracycline cardiomyopathy, we proposed a peptide preparation from the heart (HP) which is known to stimulate cardiomyocyte repair after damage caused by industrial toxins [2] and to possess an antiischemic effect [6].

In the present study we tested the possibility of correcting daunomycin-induced cardiomyopathy with the heart-derived peptide preparation.

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MATERIALS AND METHODS

White outbred male rats weighing 200-250 g were used for the experiments (Each group comprised no less then 10 animals.) Different schemes of intraperitoneal administration of daunomycin (Dm) were applied. In the first experiment, Dm was administered daily in a dose of 2 mg/ml over 7 days (total dose 14 mg/kg). Another group received HP in a dose of 0.5 mg/kg intraperitoneally during 10 days together with Dm. The animals were sacrificed on days 2 and 4 after the last injection. The second group were administered Dm once a week in a dose of 5 mg/kg overall four times (total dose 20 mg/kg). Another group received HP in a dose of 0.5 mg/kg 2-3 times per week (in all 22 injections) together with and following Dm administration. Both groups initially comprised 20 animals, which were killed on days 2 and 36 after the last Dm injection. A special group was administered only HP, and the animals were examined according to the same protocol. In the third experiment animals received four injections of 2 mg/kg Dm once a week (total dose 8 mg/kg). The HP treatment was the same as in the second experiment but more prolonged (30 injections). The animals were sacrificed on days 2, 35, and 56 after the last Dm injection. In the fourth experiment Dm (2 mg/kg per injection) was administered according to a more prolonged scheme: the 1st week, 4 injections; the 2nd week, no injections; the 3rd week,

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TABLE 1. Biochemical Parameters in Rat Heart Induced by Different Doses of Dm and after Treatment with HP

Experimental conditions	Day after cessation of administration	Number of animals	DC, nmol/g tissue	MDA, nmol/g tissue	G-6-P, μmol/g tissue	ATP, μmol/g tissue
Normal	–	13	67±14	106±16	1.03±0.12	2.63±0.18
Experiment 1:						
Dm	2nd	6	82±21	156±8**	0.87±0.14	3.21±0.18**
Dm + HP	2nd	6	116±26**	143±12**	0.66±0.27*	3.10±0.15**
Experiment 2:						
Dm	2nd	10	123±41**	128±20	0.91±0.25	2.64±0.27
Dm + HP	2nd	10	119±29**	132±14*	0.93±0.18	2.20±0.29
Dm	36th	4	96±19*	155±21**	1.53±0.43**	2.70±0.25
Dm + HP	36th	4	97±18*	102±9	0.87±0.24	2.84±0.19
HP	–	8	82±27	110±17	0.91±0.30	2.61±0.14
Experiment 3:						
Dm	2nd	5	60±24	86±27	0.97±0.29	2.73±0.36
Dm + HP	5	2nd	74±23	80±24	1.03±0.44	2.63±0.28

Note. Here and in Table 2: * – $p<0.05$, ** – $p<0.01$ in comparison with the norm.

2 injections; the 4th week, no injections; the 5th and 6th weeks, one injection each, in all 15 mg/kg cytostatic per animal. In the group treated with HP the preparation was injected in a dose of 0.5 mg/kg daily together with Dm and then during about one month after its withdrawal (a total of 40 injections). The animals were sacrificed 3-4 months after the start of the experiment and the contactility of the heart was evaluated. The content of diene conjugates (DC) [8] and malonic dialdehyde (MDA) [1] was determined in myocardial specimens preliminarily frozen in liquid nitrogen (1st and 3rd experiments). The content of ATP and glucose-6-phosphate (G-6-P) was measured with Boehringer kits according to the manufacturer's manual [4]. In the 4th experiment contractile capacity and the rhythmo-inotropic characteristics of left ventricle trabeculae were evaluated under near-isometric conditions and 1 Hz stimulation with superthreshold stimuli at 33°C in a perfusion chamber containing Krebs-Henseleit solution of the following composition (in mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.0, $MgSO_4$ 1.2, KH_2PO_4 1-2, $NaHCO_3$ 20.0, glucose 10.0, pH 7.4. Recordings were performed with a 6MKh5S mechanotron. The data were processed statistically using the Student t test.

RESULTS

In the second through the fourth experiments virtually all rats treated with Dm had more or less pronounced signs of cardiomyopathy: dilated and flaccid hearts; in the 2nd experiment round margins of the liver, ascites, and hydrothorax. The gain of body weight in rats treated with the cytostatic was delayed in comparison with normal animals, especially in the 2nd experiment. Mortality was not observed among rats in the 1st, 3rd, and 4th experiment, while in the 2nd experiment it was considerable.

In the 2nd experiment animals started to die after cessation of the Dm treatment. Some animals started to lose weight, which culminated in their death some days later. By the 36th day overall 67% animals had died in the control group and 70% in the HP-treated group. Both the single and total doses of Dm turn out to be too high and the damaging effect of the cytostatic was considerable. However, on the 36th day from termination of the Dm treatment suvivors in the control differed from those in the HP-treated group in body weight, appearance, and state of internal organs. The volume of ascitic fluid was 2-3-fold less and the liver was in better condition in treated than in non-treated animals. Moreover, unlike the treated animals, in the controls oxidative stress was observed, which manifested itself in an elevated content of MDA, the end-product of lipid peroxidation, in the heart (Table 1).

Activation of LPO was also noted at earlier time points in the 2nd experiment as well as in the 1st experiment, and the HP injections did not prevent it. Nevertheless, the normalization of the MDA content in the HP-treated vs. control survivors suggests that HP can improve repair and correct the disorders caused by anthracycline therapy. In the 3rd experiment, where the injected doses were lowered 2.5 times in comparison with the 2nd experiment, no increase of LPO products in the heart was observed (Table 1).

In regard to the energetic impacts of Dm therapy, the ATP content was virtually unchanged in all experiments except the 1st experiment,

TABLE 2. Contractile Function of Left Ventricle Trabecula 2–2.5 Months after Withdrawal of Dm (Total Dose 16 mg/kg) and after Treatment with HP

Treatment	Number of animals	After 1 h of adaptation in chamber			Rhythm-inotropic dependence of contraction at 2 Hz stimulation, (in % of initial amplitude at 1 Hz)	
		magnitude of contraction, mg	rate of contraction, mm/sec	rate of relaxation, mm/sec	after 1 h of adaptation in chamber	after action of epinephrine with subsequent washout for 30 min
Norm	8	316±71	324±97	146±44	91±9	97±4
Dm	6	160±95**	188±99*	72±35*	78±20	108±8**
Dm+HP	5	286±48	246±76	94±29*	73±15*	69±20**

where even some increase over the normal level was noted. The data on a certain stability of the ATP content are in conformity with well-known results indicating a 45% drop from the initial level of creatine phosphate without any fundamental changes in the ATP content [3]. Even though the ATP content is unchanged, this does not imply that the cardiomyocytes are supplied with energy, because the content of the main carrier of macroergic phosphate, phosphocreatine, is reduced [10]. A decreased content of G-6-P, an alternative energetic substrate, may attest to the activation of reserve pathways of energy metabolism and may be considered as a compensatory reaction (1st experiment). In contrast, the marked elevation of G-6-P in rats in the terminal state on day 36 (2nd experiment) indicates an inhibition of one of the alternative pathways for the maintenance of energetic homeostasis in the heart [9].

The study of the contractile function performed on trabeculae of the left ventricle in the 4th experiment demonstrated an impaired contractility, the strength of contractions being 2-fold lower. The treatment with heart peptides considerably improved this parameter (Table 2). Contraction and relaxation rates were also reduced in HP-treated animals but to a lesser extent than in nontreated rats (Table 2). The response to an increase of the stimulation frequency was somewhat lower in damaged than in normal muscle. The difference between the control and HP-treated groups was revealed after the action of epinephrine on the muscle. In rats treated with Dm alone, a strong stimulatory effect on contractions under high-frequency stimulation persisted after epinephrine was washed out, and the Boudich staircase became positive, which suggests either an elevated Ca^{2+} concentration in the cytoplasm or an increased sensitivity of myofibrils to Ca^{2+}, as was assumed previously [5]. However, in HP-treated rats the reaction was found to be inverse (Table 2).

Thus, HP administration to rats treated with Dm resulted in improved contractility of the heart trabeculae and reduced reaction to a Ca^{2+} excess in response to epinephrine stimulation in comparison with the control rats. HP affects the Ca^{2+} distribution between cell pools and modulates the response of the contractile apparatus to changes in the Ca^{2+} concentration. The effect of HP is probably related to improvement of the repair processes in the myocardium and its structural restoration.

The effect depends not only on the total but also on the single dose of Dm, as well as on the interval between injections. Therefore, the prevention of cytostatic overdosage is still a crucial factor in anthracycline therapy. The peptide preparation from the heart appears not to prevent the intercalation of Dm molecules into membranes and between DNA bases. Nevertheless, it may improve the structural and functional properties of the myocardium at a later stage following the withdrawal of the drug.

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