DECREASE OF MYOCARDIAL mRNA IN ADRIAMYCIN-TREATED RATS

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1. Introduction

Adriamycin is one of the most potent antitumor drugs and particularly effective against solid tumors and leukemias [1–3]. Its antitumor action has been attributed to intercalation between adjacent DNA basepairs [4] and to the thus-caused inhibitory effect on DNA replication, RNA- and protein synthesis [5,6]. Despite its very efficient inhibition of tumor growth, its clinical use has been limited by an associated, severe cardiotoxicity in form of a dose-dependent, progressive and mostly irreversible, often lethal cardiomyopathy [2,7–10]. Ultrastructurally, degeneration of various cytoplasmic structures (myofilaments, mitochondria, sarcoplasmic reticulum) and ultimately myocellular necrosis have been demonstrated both in humans and in several animal models and were held responsible for the development of the cardiomyopathy [7–9,11].

Investigations on the adriamycin cardiomyopathy have presented evidence for an inhibitory effect of adriamycin on the myocardial DNA metabolism, in particular DNA-replication and DNA-repair mechanisms [12–14]. Since the incorporation of radioactive amino acids into myocardial protein was impaired in adriamycin-treated animals [15], it was proposed that this reflects a reduced myocardial protein synthesis rate as consequence of a more direct effect of adriamycin on DNA-replication and -expression, and it was postulated that this could be responsible for the development of the adriamycin cardiomyopathy.

We have shown [16] that cyclical treatment of rats with adriamycin leads to pronounced decreases in the myocardial content of polyribosomes. We have now extended this study to an analysis of the effect of adriamycin onto the myocardial mRNA content and the activity of the myocardial pH 5 enzymes. Both parameters are severely decreased in adriamycin-treated rats, mRNA by 51.6%, the pH 5 enzymes by 35%. The specific mRNA species coding for the individual myofibrillar proteins seem to be affected to a similar degree.

2. Materials and methods

Male Sprague-Dawley rats, 150 g body weight (b.w.), fed overnight, were injected with adriamycin intraperitoneally, 10 or 15 mg/kg b.w. (control groups received 0.9% NaCl). Each group consisted of 10–15 rats. They were decapitated 7–14 days after injection of adriamycin. Hearts were pooled and polyribosomes and mRNA isolated as in [16,17].

The mRNA was translated in vitro in the mRNA-dependent reticulocyte lysate system [18], the cell-free synthesized products were separated on a 10% polyacrylamide slab-gel [19] and visualized by fluorography [20].

Myocardial contents of DNA, RNA, protein and pH 5 enzymes were determined as in [21–24].

3. Results and discussion

The effect of adriamycin on animal survival rate, body weight, heart weight, and the myocardial contents of DNA, RNA and protein are illustrated in table 1. In accordance with [14], there is a close correlation between dose of adriamycin and survival rate, with ~1/2 of the animals of the 10 mg/kg b.w. group dying within 14 days. In all subsequent experiments, adriamycin was thus given 7–14 days before decapitation. Myocardial DNA and protein contents changed only little after adriamycin treatment (table 1), myocardial total RNA and microsomal RNA somewhat more (table 1).

In contrast, adriamycin caused a severe decrease in

Table 1
Effect of adriamycin on animal survival rate, body weight and heart weight, myocardial DNA, RNA and protein contents

	Controls	Adriamycin	
		10 mg/kg body wt	15 mg/kg body wt
Animal survival rate	100%	57%	22%
Body weight (g)			
initial weight	133 ± 15.3 (11)	134 ± 17.1 (11)	133 ± 16.5 (11)
end weight	176 ± 23.4 (11)	137 ± 23.6 (11)	130 ± 36.2 (11)
Heart weight (g)	0.55 ± 0.06 (11)	0.42 ± 0.05 (11)	0.40 ± 0.09 (11)
DNA content (mg/g heart)	1.43 ± 0.12 (6)	1.31 ± 0.13 (6)	1.28 ± 0.16 (5)
RNA content			
total RNA (mg/g heart)	2.05 ± 0.18 (6)	1.71 ± 0.17 (6)	1.65 ± 0.23 (5)
microsomal RNA (μg/g heart)	703 ± 70 (11)	625 ± 113 (11)	616 ± 108 (11)
Protein content (mg/g heart)	171 ± 27 (6)	166 ± 20 (6)	164 ± 19 (5)

Values are the mean ± SEM (no. preps., each preparation containing 5–10 rats). Myocardial DNA, RNA and protein were determined essentially as in [16,21–23]. Adriamycin was injected as described in the text. Survival rates refer to the no. animals surviving 14 days after application of adriamycin (each group starting with 54 rats)

Table 2
Effect of adriamycin on myocardial contents of mRNA and polyribosomes

Animal group	mRNA (μg/g heart)	(%)	Polyribosomes (μg/g heart)	(%)
Controls	21.3 ± 4.5 (15)	100	752 ± 84 (6)	100
Adriamycin				
10 mg/kg b.w.	13.5 ± 3.4 (8)	63.4	511 ± 62 (6)	68
15 mg/kg b.w.	10.3 ± 4.2 (6)	48.4	489 ± 106 (5)	65

Values are the mean ± SEM (no. preps.). In each preparation of mRNA, 10 pooled rat hearts were homogenized in 8 vol. buffer containing 10 mM Tris–HCl (pH 7.4), 300 mM KCl, 5 mM $MgCl_2$, 250 mM sucrose, 1% Triton X-100, 100 U/ml heparin. The postmitochondrial supernatant (PMS) was obtained by centrifugation (12 500 rev./min, 20 min, 4°C) and used to isolate mRNA from the pelleted microsomes (42 000 rev./min, 70 min, 4°C in a Beckman Ti 60 rotor) by SDS–phenol extraction [27] and adsorption of the mRNA onto oligo(dT) cellulose [27]. The protein-synthesizing activity of the isolated, highly active mRNA was tested in the wheat germ system [28] and was generally of the order of 45 000 cpm/μg mRNA using the conditions in [23]. For isolation of myocardial polyribosomes, 10 pooled rat hearts were homogenized in 8 vol. buffer containing 10 mM Tris–HCl (pH 7.4), 250 mM KCl, 5 mM $MgCl_2$, 250 mM sucrose, 1% Triton X-100. The polyribosomes were isolated from the PMS essentially as described for rat liver [23,29] and were tested for protein-synthesizing activity in presence of pH 5 enzymes [23,29]

the myocardial mRNA content (table 2) from a value of 21.3 μg/g heart (controls) to 10.3 μg/g heart (15 mg adriamycin/kg b.w.), a decrease of ~50%. On a per-heart-basis, the decrease of the myocardial mRNA content is with 65% even more pronounced (11.7 μg/heart in normal rats *vs* 4.1 μg/heart in the adriamycin-treated rats; data calculated from tables 1,2). Similar sharp decreases are observed in the myocardial content of polyribosomes after adriamycin treatment as shown in table 2, in accordance with [16,17].

The observation that adriamycin causes a much greater decrease in myocardial mRNA content (table 2) than in myocardial total and microsomal RNA (table 1) suggests a predominant action of adriamycin in the nucleus rather than in the nucleolus.

Equal amounts of myocardial mRNA from normal and adriamycin-treated rats (1.6 μg each mRNA preparation) were then translated in vitro [18] and the cell-free synthesized products identified by SDS–slab-gel electrophoresis [19] and subsequent fluorography [20]. Fig.1 shows that the pattern of the cell-free synthesized myocardial proteins is practically identical for the two mRNA preparations, both qualitatively and quantitatively (fig.1, runs 3,4). This implies that the two mRNA preparations must have a nearly identical composition with respect to the individual mRNA molecules coding for those specific proteins. Thus, in the adriamycin-treated hearts, all mRNA species seem to be affected to a similar degree.

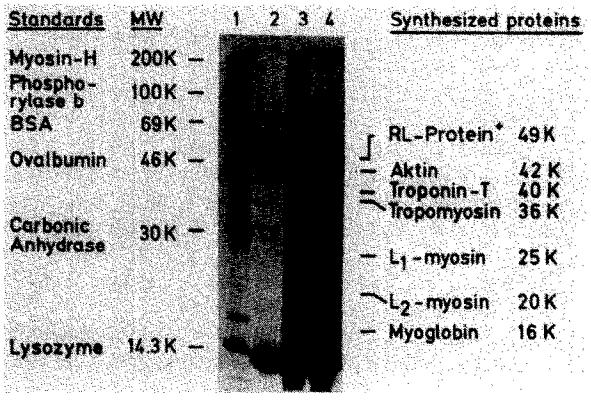

Fig.1. Translation of myocardial mRNA from normal (lane 3) and adriamycin-treated rats (lane 4) in the mRNA-dependent reticulocyte lysate system. Myocardial mRNA was isolated from normal and adriamycin-treated rat heart muscle as in section 2 and [16,17]. Of each mRNA preparation 1.6 μg was translated in the mRNA-dependent reticulocyte lysate system [18]. The cell-free synthesized products were separated on a 10% SDS–polyacrylamide slab-gel (180 V, 4.5 h) [17,19] and were visualized by fluorography [17,20]. Lane (1) contained the indicated radioactive M_r standards; (2) the reticulocyte lysate without exogenous mRNA (12 230 cpm); (3) with myocardial mRNA from normal rats (50 120 cpm); (4) with myocardial mRNA from adriamycin-treated rats (49 430 cpm). In each case, the radioactivity was contained in 1 μl of a 25 μl reaction mixture. RL-protein* refers to a protein synthesized in the reticulocyte lysate also in absence of mRNA.

By comparison with the indicated radioactive M_r standards and with non-radioactive actin, myosin, tropomyosin, troponin and myoglobin, the cell-free synthesized proteins were identified as actin, troponin-T, tropomyosin, L1-myosin, L2-myosin, and myoglobin. Various minor radioactivity bands have as yet not been identified and represent other cell-free synthesized myocardial proteins.

In [15] a reduced myocardial protein synthesis rate was demonstrated in adriamycin-treated rats. Our findings of severely decreased contents of myocardial mRNA and polyribosomes suggest that this might be

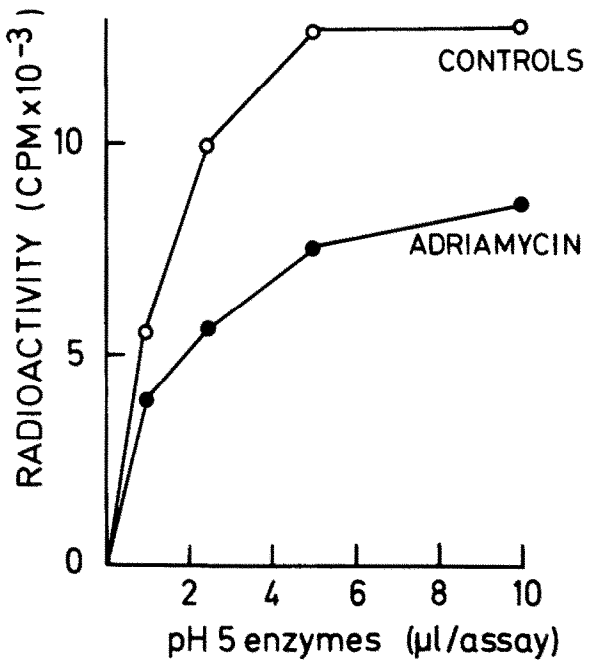

Fig.2. Effect of adriamycin on the activity of myocardial pH 5 enzymes. Myocardial pH 5 enzymes were isolated from the hearts of either normal (○) or adriamycin-treated animals (●), as described for isolation of rat liver (pH 5) enzymes [24]. Adriamycin-treatment of the animals was 10 mg/kg body wt, i.p., 2 weeks before decapitation. The activity of the pH 5 enzymes was assayed in vitro in presence of myocardial polyribosomes as in [24].

one major factor for the reduction of myocardial protein synthesis after adriamycin treatment. In addition, our results about an impairment of the activity of the myocardial pH 5 enzymes in adriamycin-treated rats (fig.2) suggest that this could also contribute to the reduced myocardial protein synthesis rates in such animals [15].

We conclude that the adriamycin caused decreases of the myocardial mRNA and polyribosome contents and the impairment of the activity of the myocardial pH 5 enzymes are the cause of the observed decrease in myocardial protein synthesis rates after treatment with adriamycin. This could be a major factor in the development of the adriamycin cardiomyopathy. Our results furthermore show that adriamycin causes an unselective decrease of all individual mRNA species coding for the major myofibrillar proteins which could suggest that the primary action of adriamycin on the DNA is similarly unselective.

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