Cholinesterases in Dexrazoxane-treated Daunorubicin Cardiomyopathy in Rabbits

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Abstract. Changes in cholinesterases activities in daunorubicin cardiomyopathy and in dexrazoxane (DRZX)-treated daunorubicin cardiomyopathy were investigated in rabbits Acetyl and butyrylcholinesterase (AChE and BuChE) were determined using Ellman's method In the serum, a significant decrease of BuChE was observed in the daunorubicin group (9 05 at the beginning and 7 15 μcat/l at the end of the experiment) After DRZX, no significant changes were found and a significant increase in BuChE was observed in the control group (10 26 – 12 38 μcat/l) AChE activity in the left and right cardiac ventricles was not significantly different between the groups while in the septum there was a significantly lower AChE activity found in the daunorubicin group only BuChE activity was significantly decreased in the left (15 64 ncat/g) and right (19 27 ncat/g) heart ventricles, in the septum and in the liver in the daunorubicin group A significant decrease in serum total protein and albumin was demonstrated only in the daunorubicin group Our results support the hypothesis about the influence of daunorubicin on protein (and enzyme) synthesis in the liver and heart A protective effect of DRZX on cholinesterases activity was observed The changes in cholinesterase activities may thus reflect their possible role in cardiomyopathy

Key words: Cholinesterases — Heart — Rabbit — Dexrazoxane — Daunorubicin

Introduction

Therapeutic use of anthracycline derivatives is limited by their side effects, especially by the cardiomyopathy (Czarnecki 1984) There have been many attempts

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to characterize or reduce the toxic effects of these drugs (Cini Neri et al 1991, Geršl and Hrdina 1994, Seifert et al 1994, Geršl et al 1995, Dorr 1996) Any information on possible influence on different systems is, therefore, important for their clinical use It has been demonstrated that the vertebrate heart contains high concentrations of acetylcholinesterase (AChE, EC 3 1 1 7) and butyrylcholinesterase (BuChE, EC 3 1 1 8) which is very probably associated with the cholinergic innervation of the heart However, there are only scarce data concerning the influence of daunorubicin on the cholinergic nervous systems in the heart (Silver 1974) Moreover, it is not known whether cardioprotective drugs can influence these enzymes involved in cholinergic nerve transmission The aim of this study was to investigate the changes of cholinesterases following dexrazoxane pretreatment in animals with daunorubicin cardiomyopathy

Materials and Methods

Animals

Medium size Chinchilla male rabbits (average weight 3 kg at the beginning of the experiment) were used The animals were allowed free access to a standard pelleted rabbit diet and tap water, and were maintained in air conditioned room The handling of the experimental animals was made under the supervision of the Ethics Committee of the Medical Faculty in Hradec Kralove, Charles University in Prague

Three groups of animals were used Drugs were administered once weekly Daunorubicin ($n = 11$) was administered over a maximum period of 9 weeks (7 10 administrations) until signs of cardiac insufficiency (i e PEP LVET ratio value above 0 5000) occurred (Geršl et al 1996b) The combination of dexrazoxane and daunorubicin (DRZX-D) was administered in five animals (10 administrations) and saline was administered in the control group ($n = 14$) The doses were selected on the basis of our previous experiments (Geršl and Hrdina, 1994) and literature data (Czarnecki 1984)

Drugs and dosages

Daunorubicin (CÉRUBIDINE, Laboratoire Roger Bellon, France, 3 mg/kg i v), dexrazoxane (CARDIOXANE, Eurocetus, the Netherlands, 60 mg/kg i p) ketamine (NARKAMON 5% inj , Spofa Czech Republic, 50 mg/kg i m), pentobarbital (NEMBUTAL, Abbott, USA), Natrium chloratum sol isotonica (Biotika, Slovakia, 1 ml/kg i v)

Intervals and sites of biochemical determinations

Collection of blood samples (from the ear artery) was performed during ketamine anaesthesia in the following intervals "1" (control value, before the first administration of the drug), "2" (before the fifth administration of the drug) and "3" (at the end of experiment, i e 5–7 days after the last administration) Animals

were killed with i v pentobarbital and tissue samples were taken Albumin and total protein were determined with standard biochemical methods using an automatic analyzer HITACHI 717 The heart was removed and dissected into the right and left ventricles and the interventricular septum Appropriate parts from approximately the same places of these areas and the liver were used for AChE and BuChE determination

Determination of AChE and BuChE activity

Tissues weighing 150 300 mg were frozen at −40 °C and homogenized (Ultra Turrax homogenizer) with 0 2 mol/l Tris-HCl buffer, pH 7 6 at 1 10 ratio In these homogenates and serum, AChE (heart) and BuChE (heart, serum, liver) activities were determined by the method of Ellman (Ellman et al 1961) using acetylthiocholine (AChE) and butyrylthiocholine (BuChE) as substrates and 5, 5′-dithiobis 2-nitrobenzoic acid as chromogen

Noninvasive polygraphic recordings

Recordings of systolic time intervals (i e , electromechanical systole "Q-2" (ms), left ventricular ejection time "LVET" (ms) and preejection period "PEP" (ms)) were obtained at the beginning and during the experiment (weekly from interval "2") On the basis of these data, PEP LVET ratio was calculated as a parameter of the heart function (Weissler and Schoenfeld 1970)

Histological examination

After the sacrification of animals the hearts were taken for histological examination Tissue blocks of the transversely sectioned left and right ventricles were immersely fixed in 10% formalin Paraffin sections (7 μm) were regularly stained with haematoxylin-eosin and Mallory's blue trichrome

Data analysis

Statistical evaluation of values was performed using a paired t-test (within one group) and by means of an unpaired t-test (comparison of different groups) for the level of significance $p \leq 0\ 05$ Values are expressed as mean ± S E M

Results

Cholinesterase activity

Serum BuChE activity was increased in the control group and decreased in the daunorubicin group (interval "3") BuChE activity in the DRZX-D group was not changed (Table 1) A significant decrease of total protein and albumin was found in the last interval studied ("3") in the daunorubicin group only (Table 1, Fig 1)
Heart Significantly lower values of AChE activity were found in the septum in the daunorubicin group only No significant differences were observed in the right and left ventricles between the groups BuChE activity in the daunorubicin group was

Table 1. Albumin, total protein and BuChE activity in the serum following daunorubicin and daunorubicin-dexrazoxane treatment

Interval		1	2	3
				s ‡
Protein (g/l)	D	67 29 ± 1 20	66 29 ± 1 49	55 45 ± 1 80
	C	63 43 ± 1 33	68 14 ± 0 77	64 50 ± 1 15
		†	s †	s
	DRZX-D	60 52 ± 0 46	62 46 ± 0 88	58 92 ± 1 71
		s		s ‡
Albumin (g/l)	D	51 36 ± 0 82	52 50 ± 1 18	40 09 ± 1 47
			‡	
	C	47 07 ± 0 71	53 36 ± 0 68	46 92 ± 0 74
		†		†
	DRZX-D	46 52 ± 0 68	49 38 ± 0 89	47 24 ± 1 47
			s	s‡
BuChE (μcat/l)	D	9 05 ± 0 48	9 03 ± 0 30	7 15 ± 0 53
				†
	C	10 26 ± 0 38	11 29 ± 0 25	12 38 ± 0 26
				s
	DRZX-D	10 18 ± 0 63	9 30 ± 0 54	9 56 ± 0 34

D = Daunorubicin group, C = Control group, DRZX-D = Dexrazoxane + daunorubicin group Values are expressed as mean ± S E M
1–3 = time intervals of the measurement (1 and 2 – before the first and the fifth administration of the drug, 3 – at the end of experiment) ‡ = significant difference ($p \leq 0\ 05$) in comparison with interval "1", s = significant difference ($p \leq 0\ 05$) in comparison with C, † = significant difference ($p \leq 0\ 05$) between D and DRZX-D

significantly lower in all parts of the heart in comparison with the control group, and in the left and right ventricles in comparison with DRZX-D group (Table 2, Figs 2 and 3)

Liver BuChE activity in the daunorubicin group was significantly reduced in comparison with the control group. In the DRZX-D group, BuChE activity was lower than in the control group but higher than that in the daunorubicin group (Table 2, Fig 3)

Noninvasive parameters of cardiac function (PEP: LVET ratio)

During the experiment, a mild, though in some intervals significant, changes were found in the control group of animals (0.3220 ± 0.0190 at the beginning and 0 3596 ± 0 0115 (117 1%) at the end of experiment). A progressive, mostly statistically significant increase in the PEP : LVET ratio (between 0.3281 ± 0.0173 and 0.6071 ± 0.0209, i.e. 180.8 %) was found during the experiment in the daunorubicin group. This increase was significantly different from values found in the control group. In the dexrazoxane-treated group with daunorubicin cardiomyopathy, a significant (though slightly less pronounced in comparison with the daunorubicin group) increase in PEP. LVET ratio was present (values between 0.3980 ± 0.0402 and 0.6010

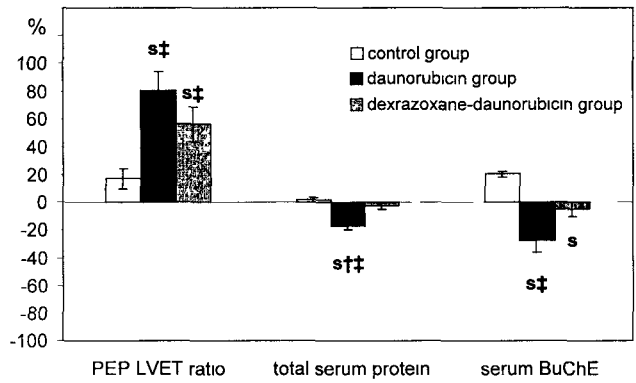

Figure 1. Changes at the end of experiment Relative values are expressed as mean ± S E M ‡ = significant difference ($p \leq 0\ 05$) in comparison with interval "1" (beginning of the experiment), s = significant difference ($p \leq 0\ 05$) in comparison with C, † = significant difference ($p \leq 0\ 05$) between D and DRZX-D

Table 2. AChE and BuChE activity in the heart and liver following daunorubicin and daunorubicin-dexrazoxane treatment

		right ventricle	left ventricle	interventricular septum	liver
				s †	
AChE	D	31 32 ± 1 24	23 09 ± 1 22	71 68 ± 1 39	–
(ncat/5 g)	C	34 66 ± 0 82	23 62 ± 0 98	88 76 ± 1 02	–
	DRZX-D	35 00 ± 0 71	22 00 ± 1 22	92 00 ± 4 38	–
		s †	s †	s	s
BuChE	D	19 27 ± 1 49	15 64 ± 2 10	53 09 ± 1 30	21 77 ± 0 56
(ncat/1 g)	C	29 85 ± 0 69	20 18 ± 0 59	99 53 ± 2 24	28 23 ± 0 51
				s	s
	DRZX-D	32 50 ± 1 43	22 00 ± 1 22	58 60 ± 2 58	23 80 ± 1 24

D = Daunorubicin group, C = Control group, DRZX-D = Dexrazoxane + daunorubicin group
Values are expressed as mean ± S E M
s = significant difference ($p \leq 0\ 05$) in comparison with C,
† = significant difference ($p \leq 0\ 05$) between D and DRZX-D

± 0.0171, i e. 156 1 %), significantly different from the control group (Fig 1).

Weight gain and premature death of animals

The weight gain in the daunorubicin group (190.2 g, i.e. increase in the body weight to 104.6%) was significantly lower in comparison with both the control group (766.7 g, i e. 124 6%) and the DRZX-D group (680.0 g, i.e. 124.6%).

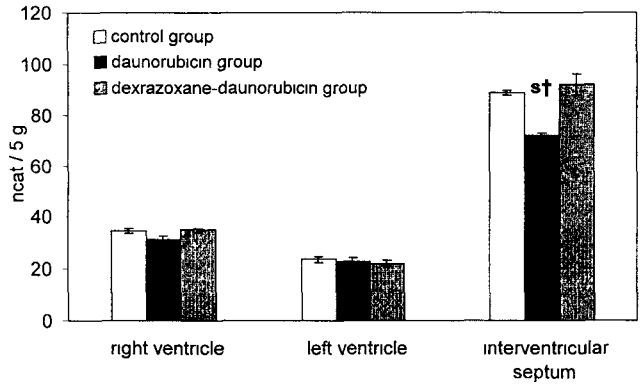

Figure 2. Acetylcholinesterase activity in the heart Values are expressed as mean ± S E M s = significant difference ($p \leq 0\ 05$) in comparison with C, † = significant difference ($p \leq 0\ 05$) between D and DRZX-D

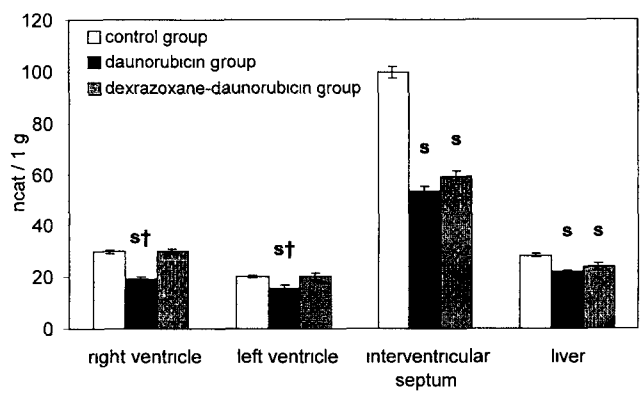

Figure 3. Butyrylcholinesterase activity in the heart and liver Values are expressed as mean ± S E,M s = significant difference ($p \leq 0\ 05$) in comparison with C, † = significant difference ($p \leq 0\ 05$) between D and DRZX-D

No premature deaths occurred in the control group and in the DRZX-D group The administration of daunorubicin induced premature deaths in 26.7% animals

Histological examination

In the control group, the myocardium showed a normal histological picture, myocytes with intensively eosinophilic cytoplasm were randomly arranged (Fig. 4). In the daunorubicin group, regressive changes of variable intensity were observed in the whole myocardium (most expressed in the ventral part of the left ventricle wall) Degeneration or necrosis of single or small groups of cardiomyocytes prevailed in

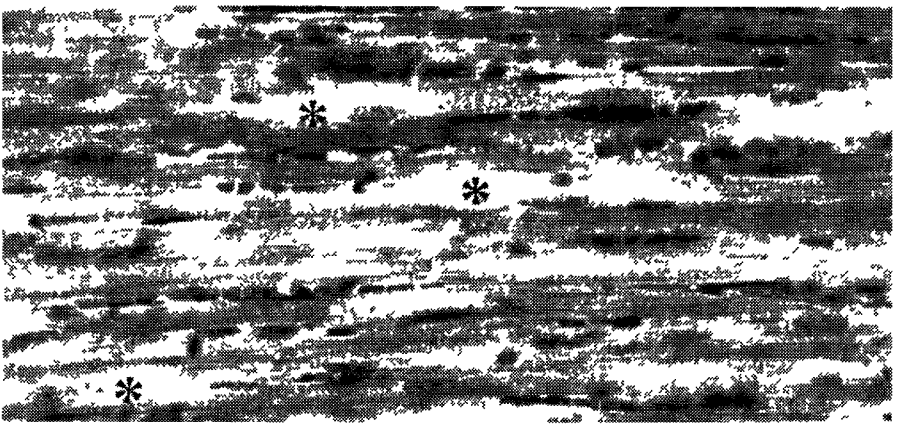

Figure 4. Rabbit left ventricle myocardium control group Normal structure of the myocardium the cardiomyocytes have centrally located pale stained nuclei which are surrounded with the cndoplasm (*) i e a part of the cytoplasm lacking cross striated myofibrils *Haematoxylin eosin, Mag* 438×

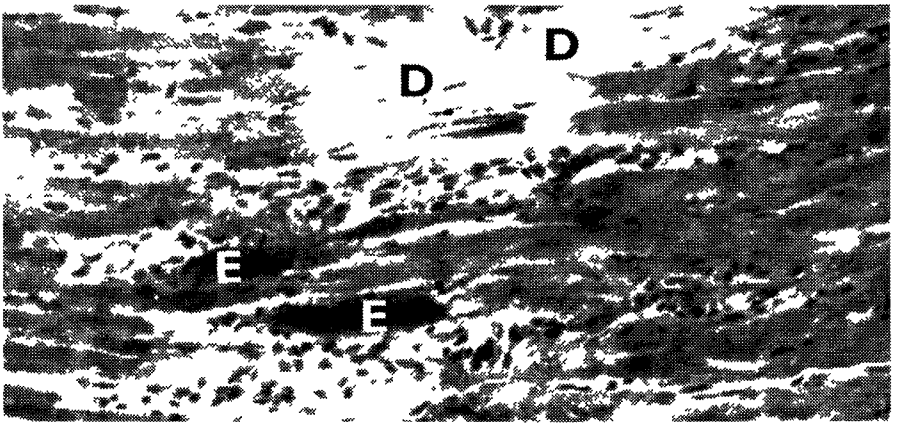

Figure 5. Rabbit left ventricle myocardium daunorubicin group Dispersed toxic dam age is found in the whole myocardium (with a maximum in the ventral part of the left ventricle wall) The degeneration (*D*) of groups of cardiomyocytes, manifested as lack of cells (empty spaces instead of cells) is frequent The cytolysis is repaired by fibrosis (i e proliferation of the connective tissue mostly from the perivascular spaces but also directly from the interstitial connective tissue) The remaining myocytes often have intensively eosinophilic cytoplasm (*E*) The damage of the right ventricle is always markedly smaller – there are mostly cells with increased eosinophilia and only scatter degenerated cells *Haematoxylin eosin, Mag* 224×

most cases with subsequent interstitial fibrosis Only several bundles of collagen fibres in intercellular spaces indicated the beginning of this reparative process Slight infiltration of adjacent stroma with leukocytes was present in a majority of animals Other damaged myocytes had mostly an intensively eosinophilic cytoplasm, single cells or groups of cells with degenerated myofibrils were often present (Fig 5) The regressive changes within the right ventricle wall were always obviously weaker The skeletal muscle tissue was of normal appearance In the DRZX-D group, the myocardial damage was mild only The only changes found were larger or smaller groups of cells or single cardiomyocytes with increased eosinophilia of the cytoplasm or with degenerated myofibrils (Fig 6)

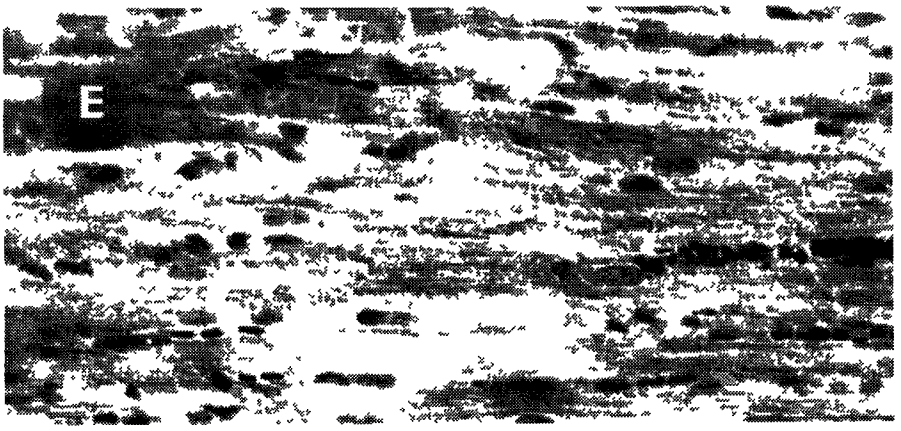

Figure 6. Rabbit left ventricle myocardium dexrazoxane + daunorubicin group Damage of the myocardium (especially in comparison with the Daunorubicin group) is mild only The larger or smaller groups of cells or single cardiomyocytes with increased eosinophilia of their cytoplasm (E) or with degenerated myofibrils (i e granulated cytoplasm) are the only changes of the myocardial structure observed *Haematoxylin eosin, Mag* 438×

Discussion

The use of anthracycline derivatives is severely limited by their dose-related cardiotoxicity including progressive cardiomyopathy and congestive heart failure (Sinha 1982, van Acker et al 1995) The rabbit model of anthracycline-induced cardiomyopathy has been frequently used for the evaluation and comparison of cardiotoxicity of various drugs (Sinha 1982, Czarnecki 1984, Reeves et al 1990, Isberg et al 1991) Systolic time intervals were recorded to evaluate changes in the heart function induced with drugs used in the present experiments Left ventricular failure is characterized by lengthening of PEP and by shortening of LVET without

marked changes in the duration of the systole (Weissler and Schoenfeld 1970, Kozak 1973), an increase of the PEP LVET ratio above 0 4 is used by some authors as a criterion of ventricular dysfunction (Gibbs et al 1984) A marked, progressive and mostly significant increase in the PEP LVET ratio was found in the daunorubicin group in our study The findings are in accordance with our previously published data (Geršl and Hrdina 1994) as well as with the data published by other authors (Sinha 1982, Czarnecki 1984, Gibbs et al 1984, 1986)

Neurotransmitters play an important role during the development of cardiomyopathy In anthracycline cardiomyopathy, the studies have mostly focused on the adrenergic nervous system (Hoyano et al 1996, Lekakis et al 1996), though possible involvement of the cholinergic system in this pathological state was also observed (Hoyano et al 1996) However, the participation of acetylcholine and the cholinergic nervous system in the genesis of cardiomyopathy has mostly been studied using cholinesterase inhibitors (Kato et al , 1989) It was clearly demonstrated that following administration of different cholinesterase inhibitors, morphological changes including cardiomyopathy were found (Kato et al 1989, Tryphonas and Clement 1994) The functional importance of BuChE activity in the heart is not yet clear BuChE was suggested to be involved in inactivation of free acetylcholine (Kutty 1980, Brown et al 1981, Bajgar 1989) and therefore reduction of BuChE activity can result in a similar effect, especially in chronic experiments, as it was demonstrated for cholinesterase inhibitors (Traina and Serpietri 1984, Bajgar 1989, 1991, Harvey 1995, Kassa and Bajgar 1995) Our previous results (Geršl et al 1996b) of normal AChE activity in the heart show very good agreement with literature data Higher concentrations of BuChE in comparison with AChE in the heart (Jbilo et al 1994, Silver 1974) and relatively low BuChE activity in the rabbit plasma and liver in comparison with other species were described previously (Jbilo et al 1994, Silver 1974, Wicki 1994) The highest AChE activity for the septum was reported by other authors (Jbilo et al 1994, Silver 1974) Analyzing the changes of cholinesterase activities in the heart, liver and plasma following daunorubicin treatment, we concluded that the changes in AChE and BuChE activities can be caused probably by the daunorubicin influence on protein (and enzyme) synthesis in the liver and heart (Geršl and al 1996b) On the other hand, the data about the influence of cardioprotective drugs on cholinesterases are not available The iron chelator dexrazoxane (ICRF-187) has been shown to reduce doxorubicin-induced cardiomyopathy and is, therefore, used in clinical praxis (Seifert et al 1994, Dorr 1996) Our results (polygraphic measurement of the heart function, biochemical and morphological data) obtained after dexrazoxane treatment are in agreement with other observations (Czarnecki 1984, Cini Neri et al 1991, Geršl et al 1996a) and also confirm a good protective effect of dexrazoxane against anthracycline cardiomyopathy (Seifert et al 1994) As it would be expected from biochemical results, a decrease of AChE activity in the septum can potentiate the action of acetylcholine (Harvey 1995) Upon DRZX-D administration, BuChE activity in the ventricles was normalised, BuChE activity in the liver and septum had a tendency to increase The same applies for BuChE in the serum an increased activity was found in the control group during

the observation period This is in agreement with literature data – an increase of BuChE in the serum was observed during time intervals used in our experiment (Kutty 1980; Brown et al 1981, Bajgar 1989, 1991). At the same time interval, a decrease of BuChE activity in the daunorubicin group was demonstrated Total protein and albumin followed a similar trend, though lower values of these parameters (but within physiological range – Suckow and Douglas 1997, probably due to the limited number of animals) were present in DRZX-D group at the beginning of the experiment It is of interest that DRZX-D group showed a tendency to normalization of cholinesterases activities as well as of protein and albumin. However, in this study, the decrease of BuChE activity was more expressed. It cannot be excluded that the decrease of both cholinesterase activities might then be related to changes in parasympathetic tonus during the development of cardiomyopathy in some laboratory animals (van Acker et al. 1995). As for cholinesterase changes (not yet described in the literature) our results suggest that dexrazoxane also has, at least partial, positive effect on changes of the cholinesterase system damaged by daunorubicin.

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