Mechanism of Cardioprotective Effect of Adenocine and Non-Glycoside Cardiotonic Drugs during Experimental Chronic Cardiac Insufficiency

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The therapeutic action of adenocine during cardiac insufficiency (heart failure) caused by ischemic (stenosis) or reperfusion (removal of ligature) injury to the myocardium prevents depletion of ATP, the major energy source for myocytes in the right and left ventricles, and a drop in NAD/NADH ratio. The development of energy shortage during heart failure cannot be eliminated by β-acetyldigoxin, levosimendan, or milrinone: the content of ATP in the right and left ventricular myocardium remained below the normal level by 28 and 29%, 37 and 33%, 32 and 28%, respectively; the NAD/NADH ratio of the energy supply system in cardiomyocytes did not return to normal. Adenocine increased the content of NAD to the normal level in both the right and left ventricles, while it remained below the normal level after administration of β-acetyldigoxin (by 24 and 19.5%, respectively), levosimendan (by 27 and 29%), and milrinone (by 26 and 24%). In contrast to β-acetyldigoxin, levosimendan, and milrinone, adenocine inhibited activity of poly(ADP-ribose*)* polymerase in both ventricles. It is concluded that adenocine directly inhibits the key enzyme triggering apoptosis; we also hypothesized that this drug activates the regulatory and signal mechanisms arresting apoptotic alterations in the myocardium during heart failure.

Key Words: *cardiac insufficiency; adenocine; apoptosis; bioenergetic insufficiency*

Cardiac insufficiency or heart failure (HF) is a multifactor pathologic process characterized by impairment of the pumping action of the heart to provide the organism with metabolic substrates; in most cases, it is accompanied by remodeling of the cardiac cavities and dramatic decrease in contractile activity of the myocardium [6,7]. Despite the molecular and subcellular mechanisms of HF development and aggravation are not fully understood [5,9], the important role of accelerated apoptotic (by hundreds times during dilated cardiomyopathy or cardiac hypertrophy) and non-programmed death of cardiomyocytes in the pathogenesis of HF is beyond doubt [5,8]. Programmed cell death (apoptosis) is a highly resistant and control-escaping cell response to various physiological and the pathological stimuli whose key trigger is poly(ADP-ribose*)* polymerase (PARP), a NAD-dependent nuclear enzyme [8-10]. Inability of myocardial mitochondria to maintain the NAD/NADH ratio, to produce sufficient amounts of ATP, and to maintain the water-electrolyte balance triggers the mechanisms of nonapoptotic cell death and the development of necrotic alterations in cardiomyocytes [5]. Consequently, our aim was to examine the possibility of arresting the onset of the processes leading to cardiomyocyte death with adenocine, β-acetyldigoxin, milrinone lactate, and levosimendan (a non-glycoside cardiotonic drug improving myocardial contractility without elevation of intracellular calcium [1-4]) during chronic HF.

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MATERIALS AND METHODS

Experiments were carried out on male and female Chinchilla rabbits (n=43) weighing 2.8-3.6 kg kept after a quarantine period on a standard diet under identical conditions in a specially equipped vivarium. A two-month graduated stenosis was simulated in 35 rabbits by a surgery of the posterior descending branch of coronary artery. All animals were randomized into 6 groups. Control group 1 (n=8) comprised virtually healthy sham-operated rabbits subjected to thoraotomy under the same conditions and at the same terms as experimental animals. Control group 2 (n=7) included rabbits with 2-month graduated stenosis subjected to 20-min clamping of the artery followed by intravenous administration of 5% glucose (50 ml) for 15 min. The injections were repeated daily during the following 4 days. Experimental groups 3-6 comprised rabbits (n=7 in each group) with 2-month graduated stenosis subjected to 20-min clamping of the artery followed intravenous infusion of 181.7 mg/kg adenocine (in 5% glucose, 50 ml over 15-20-min, group 3), 24 μg/kg levosimendan (over 10 min, group 4), 37.5 μg/kg milrinone lactate (over 15-20 min, group 5), or 0.075 μg/kg β-acetyldigoxin (over 15-20 min, group 6). The last three preparations were dissolved in 2 ml physiological saline and injected intravenously drop-by-drop. The animals were sacrificed after 4 days. The presurgery procedures and methods of assessing intracardiac hemodynamics and graduated stenosis of the coronary artery were described elsewhere [1,3]. Assays for ATP and PARP as well as the measurements of NAD/NADH ratio in cardiomyocytes were performed according to [3,4]. The data were analyzed statistically using SPSS-10 software and Student's t test.

RESULTS

In rabbits with HF, adenocine increased the maximum rates of intracardiac pressure rise (dp/dt_{max}) and fall

TABLE 1. Effects of Adenocine, β-Acetyldigoxin, Levosimendan, or Milrinone on Intracardiac Hemodynamics during HF ($M\pm m$)

Index	Control I	HF				
		control II	adenocine	β-acetyldigoxin	levosimendan	milrinone
HR, bpm	212±14	286±12*	214±8^{o}	198±8^{o}	270±12^{*x+}	254±8^{*x+}
P_{syst}, mm Hg	73±6	58±4*	69±4^{o}	72±6^{o}	72±4^{o}	80±5^{o}
dp/dt_{max}, mm Hg/sec	1740±103	1017±94**	1680±86^{o}	1476±45^{*o}	1347±104	1480±96^{o}
CWI, mm Hg/sec	540±93	409±58	687±65^{o}	587±65^{o}	909±58^{*o}	737±36^{*o}
$-dp/dt_{min}$, mm Hg/sec	1813±111	766±105**	1739±89^{o}	1345±62^{*o}	1325±95^{*ox}	1339±79^{*ox}
EDP, mm Hg	7±2	22±6*	10±2^{+}	16±3*	18±4^{*x}	20±2^{*x}

Note. p<0.01 in comparison with: *control I (normal), ^{o}control II, ^{x}HF+adenocine, $^{+}$HF+β-acetyldigoxin.

TABLE 2. Effects of Adenocine and Phosphodiesterase Inhibitors on ATP Content, Myocardial NAD/NADH Ratio, and PARP Activity in Right Ventricle during HF ($M\pm m$)

Index	Control I	HF				
		control II	adenocine	β-acetyldigoxin	levosimendan	milrinone
ATP	7.6±0.2	4.57±0.15***	6.83±0.17^{o}	5.46±0.18^{**o}	4.78±0.18^{**o+}	5.13±0.15^{**ox}
NAD	6.5±0.2	4.62±0.12**	5.97±0.13^{oo}	4.95±0.15	4.75±0.08^{**x}	4.79±0.10^{**ox}
NADH	4.7±0.2	6.10±0.08**	6.20±0.10	6.34±0.12	6.70±0.08	6.50±0.09*
NAD/NADH	1.38±0.06	0.78±0.06**	0.96±0.08^{*o}	0.78±0.07^{**ox}	0.71±0.08^{**oox}	0.74±0.09^{**oox}
PARP	0.038±0.006	0.103±0.010***	0.058±0.005^{*o}	0.088±0.006^{*ox}	0.097±0.007^{**xx}	0.089±0.010^{**xo}

Note. Here and in Tables 3: ATP content is given in μmol/g wet tissue in myocardium of the corresponding left ventricle. Concentrations of NAD and NADH are presented in nmol/mg protein, PARP activity is given in pmol/mg protein. *p<0.05, **p<0.01, ***p<0.001 in comparison with control I (norm); ^{o}p<0.05, ^{oo}p<0.01 in comparison with control II; ^{x}p<0.05, ^{xx}p<0.01 in comparison with HF+adenocine group; ^{+}p<0.01 in comparison with HF+β-acetyldigoxin group.

TABLE 3. Effects of Adenocine and Phosphodiesterase Inhibitors on ATP Content, Myocardial NAD/NADH Ratio, and PARP Activity in Left Ventricle during HF (*M*±*m*)

Index	Control I	HF				
		control II	adenocine	β-acetyldigoxin	levosimendan	milrinone
ATP	7.34±0.15	3.50±0.10*	6.89±0.11*^{o}	5.19±0.10*^{o}	3.93±0.11*	5.37±0.12*^{o}
NAD	6.70±0.20	4.69±0.09*	6.25±0.08*^{o}	5.39±0.07*^{o}	4.77±0.09*^{x}	5.10±0.09*^{ox}
NADH	4.40±0.12	5.90±0.09*	5.48±0.12*^{o}	5.67±0.14*^{o}	5.87±0.11*^{x}	5.09±0.14*^{ox}
NAD/NADH	1.52±0.14	0.79±0.09**	1.14±0.10*^{oo}	0.95±0.08**^{o}	0.81±0.10**^{x}	1.00±0.10*^{o}
PARP	0.038±0.004	0.187±0.015*	0.065±0.008*^{o}	0.098±0.009*^{o}	0.142±0.012***$^{ox+}$	0.156±0.023***$^{x+}$

($-dp/dt_{min}$) thereby decreasing the maximum intensity of myocardial work (the same pressure was developed at lower load per unit mass, there were lower number of hibernating areas and structures engaged in developing the necessary ventricular pressure, Table 1). Moreover, adenocine decreased the end-diastolic pressure (EDP) and increased the maximum rate of pressure fall in the left ventricle ($-dp/dt_{min}$). Thus, adenocine improved the systolic and diastolic functions of the heart and (especially important) restored coordination between these functions: the drug increased the correlation coefficient between dp/dt_{max} and ($-dp/dt_{min}$) to r=0.81 from r=0.31 observed during HF (p<0.001). Levosimendan and milrinone exerted less pronounced effects on central hemodynamics: specifically, EDP and intensity of myocardial strictures work did not significantly decrease. β-Acetyldigoxin increased dp/dt_{max} and ($-dp/dt_{min}$) by 45% and 76%, respectively, although these indices remained below the normal levels. Similarly, EDP did not decrease to normal. It is noteworthy that in contrast to the action of levosimendan or milrinone, β-acetyldigoxin did not significantly increase the cardiac work intensity (CWI).

The therapeutic action of adenocine during HF caused by ischemic (stenosis) and reperfusion (removal of the ligature) damage to the myocardium is based on prevention of ATP drop (the major energy source in the heart) in cardiomyocytes of left and right ventricles by 35 and 40%, respectively. Moreover, adenocine prevented the decrease in NAD/NADH ratio by 48 and 43%, respectively, in comparison with values observed in the myocardium of healthy animals (Table 1). Similar depletion of ATP and the drop of NAD/NADH ratio were observed in humans with HF [2]. The development of energy deficiency during HF was not eliminated by β-acetyldigoxin, levosimendan, or milrinone: in the myocardium of right and left ventricle, ATP level remained below the normal by 28 and 29%, 37 and 33%, and 32 and 28%, respectively. These drugs did not restore NAD/NADH ratio of the myocardial energy supply system (Tables 2, 3). Adenocine restored the normal level of NAD in both ventricles. In contrast, this parameter remained below the normal in the right and left ventricles after treatment with β-acetyldigoxin (by 24 and 19.5%), levosimendan (by 27 and 29%), or milrinone (by 26 and 24%), respectively (Tables 2 and 3).

It is especially important that in contrast to levosimendan or milrinone, adenocine inhibited PARP activity (elevated during HF) in both ventricles (Tables 2 and 3). β-Acetyldigoxin decreased PARP activity in the left ventricle by 48% relatively to the level observed after reperfusion damage to the myocardium, but produced no significant effect on this activity in the right ventricle (Tables 2 and 3). Down-regulation of ATP synthesis and decrease in the NAD/NADH ratio correlated with increase in PARP activity (r=0.081, p<0.001 and r=0.87, p<0.001, respectively). This observation attests to direct inhibition of apoptosis by adenocine. It can be hypothesized that this drug triggers the signal and control mechanisms arresting apoptotic alterations in the myocardium during HF.

The rates of pressure rise and fall in the left ventricle correlated with NAD/NADH ratio, although there was no direct correlation between the absolute contents of ATP and NAD. Although no direct dependence was established between PARP activity and the indices of intracardiac hemodynamics, the rates of pressure rise and fall in the left ventricle could nevertheless be related to PARP via the changes in NAD/NADH ratio, because this ratio is directly correlated with PARP level.

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