

ISCHEMIC AND REPERFUSIVE RELEASE OF THE ENDOGENOUS PURINES AND ITS INFLUENCE ON THE MYOCARDIAL VIABILITY DURING -ADRENERGIC BLOCKADE

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Ischemic and reperfusion release of the endogenous purines and its influence on the myocardial viability during -adrenergic blockade. K. ŻMUDKA, J. ZALEWSKI, J. DUBIEL, G. GAJOS, J. LEGUTKO, D. DUDEK, J. TRĘBACZ, A. KOCUREK, T. PRZEWŁOCKI, P. PIENIAŻEK, W. FLAMENG, H. DE GEEST. Pol. J. Pharmacol., 2001, 53, 271–282.

The aim of this study was to estimate ischemic and reperfusion release of myocardial adenosine degradation products (MADP) during -adrenergic blockade and its relation to infarct size (IS) and viable myocardium size (VM).

In a group of 24 shepherd-mongrel dogs, randomly assigned to a metoprolol (M-) and placebo-group (P-group), occlusion of the left anterior descending coronary artery (LAD) followed by reperfusion with recombinant tissue plasminogen activator was performed. Regional myocardial blood flow (MBF) was measured by the radiolabelled microsphere technique. Blood samples from aorta and great cardiac vein were collected to evaluate the concentrations of MADP. The triphenyltetrazolium chloride perfusion and fixation technique was used for infarct size measurement. MBF in the area at risk decreased in both groups during ischemia, but it was significantly higher ($p = 0.013$) in M-group. Recanalization of LAD was associated with an increase in flow in postischemic vascular bed. MBF was significantly higher ($p = 0.024$) in P-group during late reperfusion. In M-group IS was smaller ($p = 0.007$) and VM was bigger ($p = 0.007$). The correlation between arterial adenosine concentration during early reperfusion and IS ($p = 0.044$, $r = -0.588$) or VM ($p = 0.036$, $r = 0.607$) in M-group was noted. Values of net MADP balances significantly increased during early reperfusion. The correlation between reperfusion net MADP balance and IS ($p = 0.00005$, $r = 0.906$) or VM ($p = 0.016$, $r = -0.675$) in M-group was observed.

The amount of MADP released during reperfusion correlates with the IS and is inversely proportional to the area of VM. The endogenously released adenosine may have additional cardioprotective effect during -adrenergic blockade.

Key words: myocardial infarction, viable myocardium, reperfusion, ischemia, preconditioning, -adrenergic blockade, adenosine, inosine

INTRODUCTION

An episode of ischemia due to coronary occlusion lasting for 15–20 min substantially depletes high-energy phosphates and requires several days for complete recovery of hemodynamic and metabolic parameters. The amount of necrotic tissue is an important determinant of prognosis in myocardial infarction [7, 53].

Early diagnosis of myocardial infarction is essential for effective treatment. Biochemical markers of myocardial necrosis such as creatinine kinase MB mass, myoglobin, troponin T and I are important for confirmation of ECG and clinical diagnoses [15, 23, 38]. Myocardial purine release may be another useful marker of ischemic and reperfusion damage [9]. The balance of energy metabolism is expressed by the phosphorylation state of cytosolic nucleotides. This variable cannot be measured directly because of nucleotide compartmentalization, but it can be estimated by the release of purine catabolites [58].

Reperfusion (thrombolytic and/or angioplasty) therapy is an integral component of current medical practice. A reduction in the extent of necrosis by early reperfusion has been demonstrated in many studies [27, 50]. Deleterious effects of acute blood flow restoration have also been described. During the recovery period, myocardial cells are vulnerable to stresses produced by reflow and can be irreversibly damaged [29, 47]. Adenosine is also taken into account among many agents, which could attenuate the myocardial reperfusion injury. The Acute Myocardial Infarction Study of Adenosine (AMISTAD) demonstrated that intravenously administered adenosine given as an adjunctive to immediate reperfusion therapy improved myocardial salvage and reduced infarct size in acute myocardial infarction patients [37].

The aim of this study was to estimate ischemic and reperfusory release of myocardial adenosine degradation products during α -adrenergic blockade and its relation to the infarct size and to the amount of viable myocardium size.

MATERIALS and METHODS

Animal preparation

Experiments were performed on 24 shepherd-mongrel dogs of both sexes, weighing between 17 and 30 kg, which were fasted for 12 h prior to the

procedure. All dogs were premedicated by intramuscular injection of a combination of fluanisone and fentanyl citrate (Hypnorm) at a dose of 250 mg/kg. Anesthesia was induced by slow bolus *iv* injection of sodium pentobarbital (Nembutal) at a dose of 30 mg/kg and maintained by continuous infusion of 6 mg/kg/h of Nembutal in Ringer's solution at a rate of 30 ml/h. The dogs were intubated with a cuffed endotracheal tube and ventilated with a Bird respirator connected to a blender giving the inhalation of a 21% oxygen/gas mixture. The respiratory rate of 15–18/min was readjusted as necessary to stabilize levels of arterial pO_2 , pCO_2 and pH within the range of normal. The rectal temperature of the animals was continuously monitored and kept within the physiological limits. Appropriate catheters were introduced into aorta, great cardiac vein and peripheral veins for administration of drugs, withdrawal of blood samples, coronary flow measurements and pressure monitoring.

Experimental protocol

After insertion of all catheters, a period of stabilization was started. During this time, measurements of arterial oxygen pressure, carbon dioxide pressure, pH and rectal temperature were taken and, if necessary, corrected to the normal values. After a baseline period of 30 min, coronary blood flow was measured and arterial and cardiac vein blood samples were drawn for measurement of baseline levels (B) of myocardial adenosine degradation products (MADP). Thrombotic occlusion of the medial part of the left anterior descending coronary artery (LAD) was provoked by transarterial insertion of a copper coil [11, 30, 54]. Fifteen minutes after angiographic confirmation of the coronary artery occlusion, the dogs were randomly assigned to a treatment- and placebo-group. In 12 animals (the M-group) metoprolol at 0.3 mg/kg was given *iv* followed by a continuous infusion of 0.3 mg/kg/h. In the other 12 animals (the P-group) placebo (saline) was infused. After occlusion period of 60 min (O) coronary blood flow was measured and blood samples taken. Immediately thereafter, *iv* infusion of recombinant tissue plasminogen activator (rt-PA) at the rate of 10 mg/kg/min was initiated and maintained for 30 min. At the end of the rt-PA infusion coronary artery patency was verified angiographically, the copper coil removed, coronary blood flow determined and blood samples taken (early reperfusion period, R1). The last blood samples and blood

flow measurements (late reperfusion period, R2) were done 1 h after the confirmation of coronary artery patency. Those last measurements were followed by coronary artery angiogram to confirm persistent vessel patency and by a coronary sinus angiogram to evaluate the correct position of the catheter in the great cardiac vein. Finally, the dogs were sacrificed by an intravenous solution of saturated potassium chloride, the thorax opened, the position of the great cardiac vein catheter confirmed and the heart was removed.

The animal studies were done in accordance with the Helsinki Declaration and were approved by the Ethics Committee of the Catholic University in Leuven.

Biochemical analyses

Blood samples were obtained simultaneously from the aorta and great cardiac vein. For measurements of adenosine, inosine, hypoxanthine, xanthine and uric acid concentrations, samples were taken using syringes filled with transport inhibitor (Janssen Pharmaceutica) and immediately centrifuged in a cool place (4°C). Analyses were made by single-run high performance liquid chromatography [57]. Myocardial net adenosine, inosine, hypoxanthine, xanthine and uric acid balance (MB) were calculated according to the following formula: $MB = (V-A) \times MBF$ [mol/min/100 g] where: A – concentrations of adenosine, inosine, hypoxanthine, xanthine and uric acid in ascending aorta [mol/ml]; V – concentrations of adenosine, inosine, hypoxanthine, xanthine and uric acid in great cardiac vein [mol/ml]; MBF – myocardial blood flow in the area at risk [ml/min/100g]. Reperfusion net adenosine, inosine, xanthine, hypoxanthine and uric acid balances were calculated as the sum for early and late phase of reperfusion.

Regional myocardial blood flow

Regional myocardial blood flow was measured using the radiolabelled microspheres technique. Through a pig tail 7F catheter, Du Pont Nen-Trac microspheres labelled with four different radioactive nuclides: Cerium-141, Ruthenium-103, Niobium-95, Tin-113 were injected into the left ventricular cavity. The mean diameter of the microspheres was 15.5 ± 0.1 μ m. Microspheres spread proportionally in the blood flow. Blood was withdrawn from the femoral artery through a pump with the speed of 8.69 ml/min to serve as a reference

sample. Myocardial blood flow (MBF) was calculated according to the following formula: $MBF = 8.69 \times A_M/A_B$ [ml/min/100g], where A_M – myocardial sample activity; A_B – reference arterial blood sample activity. The radioactivity of the reference blood sample and myocardial tissue was determined by a counter (analyzer model 45, Molsgaard Medical, Horsholm, Denmark) connected to a ND 680 programmable analyzer/computer system (Nuclear Data GmbH, Frankfurt/Main). Regional myocardial blood flow was calculated as a sum of flows in each myocardial tissue sample belonging to the given region [8, 17, 26, 34].

Infarct size

The triphenyltetrazolium chloride perfusion and fixation technique was used to measure the infarct size. After removal of the heart, the left anterior descending coronary artery was cannulated distal to the occlusion site and perfused with the solution of triphenyltetrazolium chloride at a pressure of 80 to 120 mmHg in order to stain the vascular bed supplied by the left anterior descending artery distal to the induced occlusion. At the same time the ostia of both coronary arteries were also cannulated and perfused at an identical pressure with a solution of Evans blue to counterstain the remaining vascular bed. The left ventricle was dissected free and cut parallelly to its base into 1-cm thick slices. Colour pictures of the upper side of each slice were made and the contours of the left ventricle, the area at risk, the non-risk area viable and necrotic areas were reproduced with black ink on transparent plastic sheets. The infarct 1.0 image processing software (S.I.D. Janssen Pharmaceutica N.V.) was used for planimetric assessment of the infarct size [18, 19, 35, 43]. Infarct size was expressed as percentage of the left ventricle (I/LV) and as percentage of the area at risk (I/P). The size of viable myocardium was expressed as percentage of the left ventricle (P-I/LV) and as percentage of the area at risk (P-I/P).

Statistical analysis

Data expressed as means and standard deviations were stored in Excel database. Statistical analysis was performed using Statistica Version 5 '97 Edition program. Student's *t*-test for unpaired variables was used for comparison of differences between both treatment (placebo and metoprolol) groups and four time intervals (baseline, occlusion,

Table 1. Infarct size, area at risk and viable myocardium size

Group		LV [cm ²]	P [cm ²]	I [cm ²]	P-I [cm ²]	P/LV [%]	I/LV [%]	I/P [%]	P-I/LV [%]	P-I/P [%]
M	X	126.715	40.132	10.045	30.087	31.3	7.4	20.1	23.9	79.9
	SD	29.118	15.401	12.471	9.963	0.095	0.090	0.204	0.067	0.204
M vs. P	p	NS	NS	NS	NS	NS	0.05	0.007	0.03	0.007
P	SD	19.985	12.872	8.767	9.879	0.079	0.058	0.173	0.066	0.173
	X	136.226	42.433	18.262	24.171	31.1	13.5	42.9	17.6	57.1

Abbreviations: LV – area of the left ventricle, P – area at risk, I – infarct size, P-I – viable myocardium, P/LV – area at risk expressed as percentage of the left ventricle, I/LV – infarct size expressed as percentage of the left ventricle, I/P – infarct size expressed as percentage of area at risk, P-I/LV – viable myocardium size expressed as percentage of the left ventricle, P-I/P – viable myocardium size expressed as percentage of area at risk, M – metoprolol-group, P – placebo-group, X – mean value, SD – standard deviation, p – probability value

reperfusion R1, reperfusion R2). Regression analysis between net MADP balance, concentration of MADP and infarct size or viable myocardium size was performed. A probability value of less than 0.05 was considered to be statistically significant.

RESULTS

Infarct size and viable myocardium size

The area at risk expressed as percentage of total area of the left ventricle (P/LV) was the same in both compared groups. Infarct size expressed as percentage of total area of the left ventricle (I/LV) ($p = 0.05$) and as percentage of area at risk (I/P) ($p = 0.007$) was smaller in the M-group. The size of viable myocardium expressed as percentage of total area of the left ventricle (P-I/LV) ($p = 0.03$) and as percentage of the area at risk (P-I/P) ($p = 0.007$) was significantly higher in the M-group (Tab. 1).

Regional myocardial blood flow

Regional myocardial blood flow in the area at risk was the same in P- and M-group during basal conditions. Blood flow decreased in both groups during ischemia, but was significantly higher ($p = 0.013$) in the M-group at 60 min following LAD occlusion (Tab. 2). Recanalization of LAD was associated with an increase in flow in the postischemic vascular bed. Regional myocardial blood flow during late reperfusion was significantly higher ($p = 0.024$) in the P-group (Tab. 2). There was no

Table 2. Regional myocardial blood flow in the area at risk [ml/min/100 g]

Group		B	O	R1	R2
M	X	58.82	18.7	98.4	37.22
	SD	48.79	7.59	29.29	10.21
M vs. P	p	NS	0.013	NS	0.024
P	SD	7.14	6.1	27.3	15.19
	X	45.57	11.06	119.56	50.05

Abbreviations: B – baseline, O – occlusion, R1 – early reperfusion, R2 – late reperfusion, M – metoprolol-group, P – placebo-group, X – mean value, SD – standard deviation, p – probability value

correlation between arterial concentration of adenosine and blood flow in the area at risk during early and late reperfusion ($r = 0.302$, $p = 0.341$).

Adenosine degradation products

Arterial adenosine and inosine concentrations did not change during ischemia and reperfusion. Hypoxanthine, xanthine and uric acid levels increased in both groups to the highest mean values during early reperfusion (Tab. 3). A gradual increase in mean MADP concentrations in great cardiac vein blood was noted. Highest levels were observed during early reperfusion. During late reperfusion MADP concentrations in great cardiac vein were within the range of baseline values (Tab. 4).

A negative correlation between arterial adenosine concentration during early reperfusion and infarct size expressed as percentage of total area of the left ventricle (I/LV) in the M-group was noted ($r = -0.588$, $p = 0.044$). There was also a positive correlation between arterial adenosine concentra-

tion during early reperfusion and viable myocardium expressed as percentage of total area of the left ventricle (P-I/LV) in the M-group ($r = 0.607$, $p = 0.036$). Therefore, high arterial adenosine concentration was associated with smaller infarct size and higher area of viable myocardium (Fig. 1 and 2)

Table 3. Arterial concentrations of adenosine degradation products [mol/ml]

		B	O	R1	R 2	
Adenosine (M vs. P)	M	X	0.083	0.079	0.063	0.078
		SD	0.094	0.093	0.078	0.160
		p	NS	NS	NS	NS
P	SD	0.129	0.164	0.185	0.072	
	X	0.108	0.107	0.125	0.053	
Inosine (M vs. P)	M	X	0.231	0.299	0.433	0.308
		SD	0.365	0.391	0.426	0.398
		p	NS	NS	NS	NS
P	SD	0.192	0.217	0.263	0.244	
	X	0.151	0.172	0.303	0.191	
Hypoxanthine (M vs. P)	M	X	0.035	0.078	0.295	0.034
		SD	0.050	0.066	0.340	0.054
		p	NS	0.0172	NS	NS
P	SD	0.087	0.116	0.284	0.033	
	X	0.052	0.022	0.338	0.014	
Xanthine (M vs. P)	M	X	0.143	0.169	0.636	0.181
		SD	0.247	0.216	0.740	0.344
		p	NS	NS	NS	NS
P	SD	0.097	0.157	0.409	0.153	
	X	0.052	0.133	0.603	0.061	
Uric acid (M vs. P)	M	X	3.879	4.398	5.630	5.242
		SD	1.242	1.025	1.587	2.458
		p	NS	NS	NS	NS
P	SD	1.350	1.071	1.648	0.892	
	X	4.383	4.428	6.323	4.851	

For abbreviations see Tables 1 and 2

Table 4. Concentrations of adenosine degradation products in blood of great cardiac vein [mol/ml]

		B	O	R1	R2	
Adenosine (M vs. P)	M	X	0.070	0.0992	0.170	0.0617
		SD	0.093	0.125	0.145	0.119
		p	NS	NS	NS	NS
P	SD	0.071	0.089	0.363	0.072	
	X	0.046	0.067	0.331	0.061	
Inosine (M vs. P)	M	X	0.268	1.470	7.533	0.392
		SD	0.377	0.739	8.056	0.430
		p	NS	NS	NS	NS
P	SD	0.190	0.994	14.062	0.311	
	X	0.148	1.963	13.049	0.233	
Hypoxanthine (M vs. P)	M	X	0.044	1.38	3.668	0.227
		SD	0.056	0.483	3.987	0.381
		p	NS	NS	NS	NS
P	SD	0.033	1.056	4.772	0.247	
	X	0.014	1.758	4.794	0.202	
Xanthine (M vs. P)	M	X	0.167	1.038	1.929	0.411
		SD	0.249	0.340	1.930	0.490
		p	NS	NS	NS	NS
P	SD	0.141	0.778	1.776	0.186	
	X	0.083	1.056	1.975	0.183	
Uric acid (M vs. P)	M	X	4.21	4.79	7.92	5.131
		SD	1.074	0.870	4.367	1.595
		p	NS	NS	NS	NS
P	SD	1.336	0.966	4.385	1.146	
	X	4.408	5.247	8.956	5.231	

For abbreviations see Tables 1 and 2

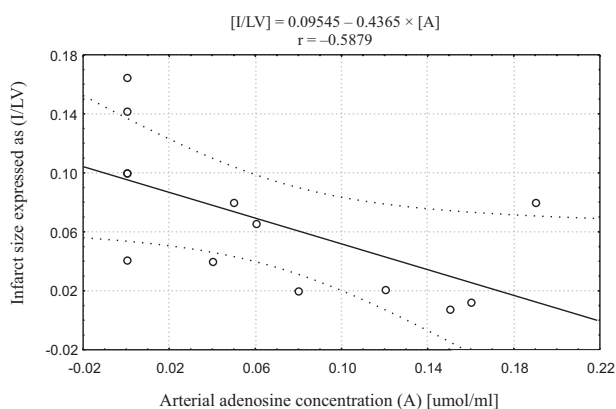


Fig. 1. The correlation between arterial adenosine concentration during early reperfusion (A) [mol/ml] and infarct size (expressed as percentage of total area of the left ventricle (I/LV)) in the M-group ($r = -0.5879$, $p = 0.0444$); r – correlation coefficient, p – probability value

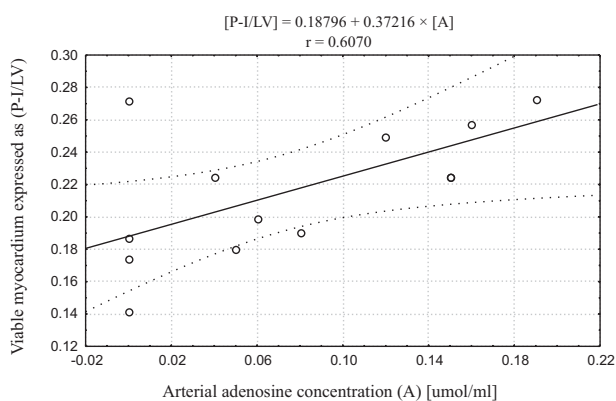


Fig. 2. The correlation between arterial adenosine concentration during early reperfusion (A) [mol/ml] and viable myocardium (expressed as percentage of total area of the left ventricle (P-I/LV)) in the M-group ($r = 0.6070$, $p = 0.0363$); r – correlation coefficient, p – probability value

in the M-group. There was no similar correlation in the P-group. The correlation between inosine concentration during early reperfusion and infarct size expressed as percentage of total area of the left ventricle (I/LV) in both groups was not observed.

The net MADP balances were close to zero during baseline conditions, 1 h after occlusion and during late reperfusion. Significantly, increased values of the calculated net MADP balances were observed during early reperfusion (Tab. 5). A positive correlation between reperfusion net adenosine, inosine, xanthine, hypoxanthine and uric acid balances and the infarct size ((I/LV) and (I/P)) was observed in the M-group (Tab. 6, Fig. 3). In the M-group, the

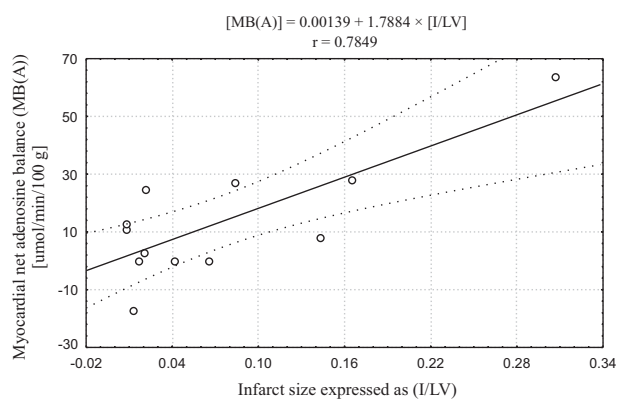


Fig. 3. The correlation between infarct size (expressed as percentage of total area of the left ventricle (I/LV)) and myocardial net adenosine balance for the sum of early and late phase of reperfusion (MB(A)) [mol/min/100 g] in the M-group ($r = 0.785$, $p = 0.0025$); r – correlation coefficient, p – probability value

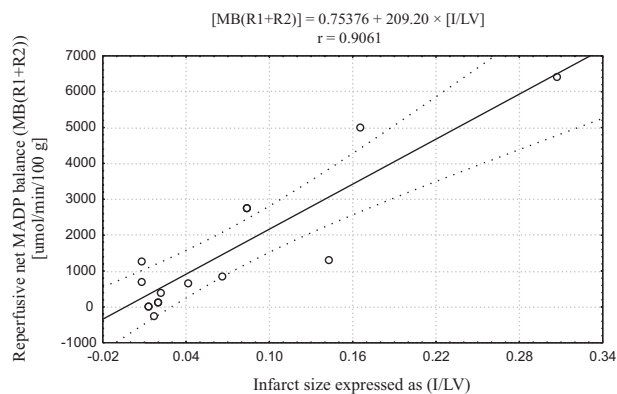


Fig. 4. The correlation between infarct size (expressed as percentage of total area of the left ventricle (I/LV)) and net MADP balance for the sum of early and late phase of reperfusion (MB(R1+R2)) [mol/min/100 g] in the M-group ($r = 0.906$, $p = 0.00005$); r – correlation coefficient, p – probability value

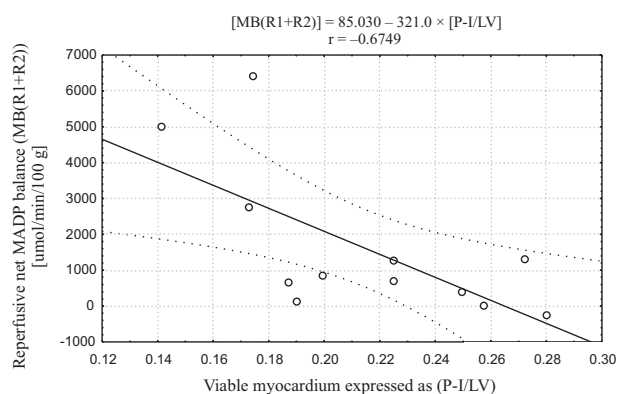


Fig. 5. The correlation between viable myocardium (expressed as percentage of total area of the left ventricle (P-I/LV)) and net MADP balance for the sum of early and late phase of reperfusion (MB(R1+R2)) [mol/min/100 g] in the M-group ($r = -0.675$, $p = 0.016$); r – correlation coefficient, p – probability value

Table 5. Net MADP balances [mol/min/100 g]

		B	O	R1	R2	R(1+2)	
M	X	0.11	0.09	12.03	-0.69	11.41	*
	SD	4.31	1.39	18.5	4.95	0.203	
Adenosine (M vs. P)	p	NS	NS	NS	NS	NS	
	P	SD	6.25	0.94	50.74	2.52	51.03
	X	-2.89	-0.21	27.97	0.3	28.27	*
M	X	3.32	21.52	787.21	2.79	7900	*
	SD	0.066	0.174	1005.01	7.31	1087.2	
Inosine (M vs. P)	p	NS	NS	NS	NS	NS	
	P	SD	1.82	15.89	1661.28	8.82	16.652
	X	-0.8	18.75	1585.74	3.2	1588.94	*
M	X	1.78	24.4	391.08	6.46	397.54	*
	SD	6.09	14.49	505.11	11.2	513.93	
Hypoxanthine (M vs. P)	p	NS	NS	NS	NS	NS	
	P	SD	3.28	15.86	532.11	15.5	535.13
	X	-1.43	18.34	548.68	10.62	559.3	*
M	X	0.9	16.01	147.47	7.86	155.33	*
	SD	11.85	7.98	180.42	18.07	193.1	
Xanthine (M vs. P)	p	NS	NS	NS	NS	NS	
	P	SD	8.52	9.59	168.77	7.96	170
	X	-5.75	9.36	167.23	6.91	174.14	*
M	X	31.58	7.55	265.29	4.64	269.93	*
	SD	61.86	14.93	417.5	42.07	405.63	
Uric acid (M vs. P)	p	NS	NS	NS	NS	NS	
	P	SD	38.58	8.05	465.93	34.9	470.61
	X	3.17	8.85	338.56	20.48	358.94	*
M	X	37.74	69.86	1603.13	21.11	1624.24	*
	SD	74.25	38.74	2067.85	51.74	2088.02	
Summary (M vs. P)	p	NS	NS	NS	NS	NS	
	P7	SD	54.43	45.91	2675.32	48.26	2691.81
	X	0.91	55.13	2668.21	41.57	2709.76	*

For abbreviations see Tables 1 and 2; R(1+2): the sum of early and late phase of reperfusion, * p < 0.05 – significant difference between O and R1

correlation between reperfusion myocardial net adenosine degradation products balance and infarct size ranked in order: uric acid, hypoxanthine, xanthine, inosine and adenosine (Tab. 6). A positive correlation (r = 0.906, p = 0.00005) between reperfusion net adenosine degradation products balance and infarct size (I/LV) was observed in the M-group (Fig. 4). In this group, a negative correlation (r = -0.675, p = 0.016) between reperfusion net MADP balance and area of viable myocardium (P-I/LV) was also noted (Fig. 5).

DISCUSSION

The effects of cardiodepressant mediators released after myocardial ischemia are counteracted by a time-dependent release of catecholamines. Endogenous cardiac adenosine having, as one of its effects, anti- adrenergic action, attenuates the modulatory effects of catecholamines [55]. Short-lasting hypoxia causes a release of both norepinephrine and adenosine in sufficient quantities so that either can completely precondition the heart [10]. We suppose that cardioprotective effect observed in our experiment as the decrease in the infarct size could be due to endogenous adenosine and its anti- adrenergic effect.

Regional myocardial perfusion can be measured by a variety of tracer techniques. In the experimental settings, the use of radioactive microspheres, has become the gold standard for the measurement of regional myocardial blood flow [25]. In our study, the application of metoprolol was associated with bigger collateral blood flow in the area at risk during occlusion and greatly decreased myocardial flow during late reperfusion. The regional blood flow in the area at risk in the M-group underwent lesser fluctuations.

Blood samples from the aorta and the great cardiac vein were collected to measure the concentrations of MADP by high performance liquid chromatogra-

Table 6. The correlation between net MADP balances for the sum of early and late phase of reperfusion (R1+R2) [mol/min/100g] and infarct size (IS) expressed as I/LV or I/P

Group	IS	Correlation between IS and net MADP balances					
		MB(A)	MB(I)	MB(H)	MB(X)	MB(UA)	
M	I/LV	r	0.785	0.859	0.888	0.916	0.928
		p	0.0025	0.0003	0.0001	0.00003	0.00001
	I/P	r	0.735	0.902	0.908	0.926	0.886
		p	0.007	0.00006	0.00005	0.00002	0.0001
P	I/LV	r	0.432	0.560	0.582	0.579	0.526
		p	NS	NS	0.047	0.049	NS
	I/P	r	0.411	0.578	0.609	0.550	0.348
		p	NS	0.049	0.036	NS	NS

For abbreviations see Tables 1 and 2; r – correlation coefficient, p – probability value, MB(A) – myocardial net adenosine balance, MB(I) – myocardial net inosine balance, MB(H) – myocardial net hypoxanthine balance, MB(X) – myocardial net xanthine balance, MB(UA) – myocardial net uric acid balance

phy. Venous drainage of the left main coronary artery occurs by the coronary sinus, anterior cardiac veins and Thebesian vessels. 50–85% of the coronary inflow reaches the coronary sinus [24, 44, 49]. Owen et al. [46] found that blood sampled in the anterior or distal part of the great cardiac vein originated predominantly from the myocardium supplied by the anterior descending artery. Circumflex venous outflow does not pass retrogradely through venous inter-connections. Hence, blood sampled near the origin of the great cardiac vein represents, first of all, anterior descending artery outflow. We observed the evidence of myocardial washout of adenosine degradation products in both groups when blood flow was restored during early reperfusion. The total amount of the released adenosine degradation products during reperfusion tended to be higher in the P-group. During late reperfusion net MADP balances were within the range of baseline values.

The significance of adenosine degradation products as an indicator of myocardial ischemia is controversial. Since adenosine fulfills key functions in the regulation of cardiac metabolism, its sensitivity as a marker of tissue ischemia has been investigated in many studies in relation to other metabolites. With periods of coronary occlusion longer than 30 s, the relative rank order of the sen-

sitivity in indicating myocardial ischemia of the purines increased as follows: uric acid, hypoxanthine, lactate and adenosine. Coronary sinus concentration of adenosine is quantitatively sufficient to be responsible for some of the changes in coronary blood flow occurring during reactive hyperemia [5]. To determine whether the release and concentrations of purine degradation products relate to infarct size, aortic and great cardiac vein blood samples were collected. In our study, a positive correlation between reperfusion net adenosine, inosine, xanthine, hypoxanthine and uric acid balances and an infarct size were observed in the M-group. In the M-group the correlation between the amount of released adenosine degradation products and infarct size ranked in order: uric acid, hypoxanthine, xanthine, inosine and adenosine. A positive correlation between reperfusion net MADP balance and infarct size was observed in

the M-group. Also in this group, a negative correlation between reperfusion net MADP balance and viable myocardium could be seen.

The triphenyltetrazolium chloride perfusion and fixation technique was used to measure infarct size. The area at risk in both groups was similar. Infarct size expressed as percentage of the total area of the left ventricle and as percentage of the area at risk was smaller in the M-group. The area of viable myocardium expressed as percentage of total area of the left ventricle and as percentage of area at risk was significantly higher in the M-group.

Higher arterial adenosine concentration during early reperfusion in the M-group was associated with significantly smaller infarct size and with significantly higher viable myocardium size. This observation could confirm the cardioprotective effect of adenosine. There was no correlation between arterial concentration of adenosine and blood flow in the area at risk during early reperfusion.

Adenosine is endogenous vasodilator that may be an important mediator of coronary autoregulation [6]. The endogenously released adenosine may play a role in the limitation of myocardial ischemia-reperfusion injury through its ability to cause vasodilatation, anti-adrenergic effect, inhibition of neutrophil function and reduction of neutrophil-mediated endothelial damage [12, 13, 51, 56]. The

intracoronary administration of adenosine has been shown in animal models to improve myocardial blood flow and reduce infarct size [45]. Endogenous and exogenous adenosine may enhance myocardial tolerance to ischemia partly *via* the modulation of glucose metabolism [31]. The beneficial effects of adenosine on the ischemic myocardium are mediated by interaction with adenosine A₁ receptor [32]. The A₃ adenosine receptor agonist affords a powerful protection against both reversible (stunning) and irreversible (infarction) injury during acute myocardial ischemia and reperfusion by a protein kinase C mediated pathway [4, 36]. Metabolic interventions capable of preventing ventricular reversible dysfunction (stunning) or accelerating its functional recovery may have potential clinical importance. The simultaneous inhibition of nucleoside transport and adenosine deaminase leads to the accumulation of endogenous adenosine and protection of the myocardium against stunning. The augmentation of endogenous adenosine (without exogenous adenosine administration) represents an effective therapeutic approach to the alleviation of reversible postischemic dysfunction [1, 2, 59]. Moreover, the enhancement of interstitial fluid adenosine to the extent provided by adenosine deaminase inhibition alone is not sufficient to protect the heart as seen in ischemic preconditioning [39, 52].

Treatment with intravenous adenosine and lidocaine during acute myocardial infarction is safe, reduces the area at risk to 45%, and thus has potential for myocardial protection [21, 22]. Marzilli et al. [40] studied the application of adenosine in patients undergoing primary angioplasty in acute myocardial infarction. Although residual coronary stenosis was similar in both groups, coronary flow was better and “no-reflow” was less common in adenosine-treated patients. Intracoronary adenosine as an adjunct to primary angioplasty ameliorates flow, prevents the no-reflow phenomenon, improves ventricular function and is associated with a more favorable clinical course [3, 41]. The AMISTAD trial demonstrated that intravenously administered adenosine given as an adjunctive to immediate reperfusion therapy improved myocardial salvage and reduced infarct size in patients with acute myocardial infarction [37]. This benefit was limited only to the patients with anterior wall infarction. There was a 71% reduction in infarct size in this subgroup. The rates of mortality, reinfarction and chronic heart failure were similar in both treatment

groups. Kerensky et al. [28] demonstrated that the first balloon inflation during coronary angioplasty provided a preconditioning stimulus leading to decreased ischemia during subsequent balloon inflations. It was also shown that intracoronary adenosine administration prior to the first balloon inflation during percutaneous transluminal coronary angioplasty modified the preconditioning response. Leeser et al. [33] proved that pretreatment before brief coronary occlusion during coronary angioplasty with adenosine, resulted in substantially less ST-segment shift and chest pain. The administration of adenosine during cardiopulmonary bypass resulted in less need for inotropic support and improved regional wall motion [20, 42]. These data suggest that adenosine plays a role in ischemic preconditioning in humans [28].

Inosine is a positive inotropic agent and dilates coronary blood vessels. During ischemia, inosine infusion increases blood flow, resulting in decreased myocardial damage. Inosine was protective against ATP loss during ischemia and improved functional recovery on reperfusion [16]. Inosine decreased the area of ischemic injury and reduced an increase in R-wave voltage induced by acute coronary occlusion in the pig [14]. However, according to Powers et al. [48] inosine pretreatment did not significantly affect the time course of post-ischemic functional recovery of rat myocardium. In our study, the correlation between early reperfusion aortic inosine concentration and infarct size was not observed.

To conclude, it seems that the amount of the purine degradation products released during ischemia and reperfusion correlates with the infarct size and is inversely proportional to the area of viable myocardium. Also we suppose that endogenously released adenosine during reperfusion may have protective effect on myocardium.

CONCLUSIONS

1. The amount of adenosine degradation products released during reperfusion correlates with the infarct size and is inversely proportional to the area of viable myocardium.

2. The endogenously released adenosine may have additional cardioprotective effect during α -adrenergic blockade.

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