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Short communication

Adenosine deaminase activity in blood of patients with stable angina pectoris

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The activity of adenosine deaminase (EC.3.5.4.4) in granulocytes and lymphocytes of patients with stable angina pectoris was lower by about 27% and 24%, respectively as compared with control group, whereas these values in erythrocytes and blood plasma were at the normal level.

Adenosine is a potent coronary vasodilator that improves myocardial perfusion in the ischemic myocardium [1–3]. Several studies have demonstrated a high correlation between cardiac formation of adenosine and myocardial oxygen uptake or the degree of hypoxia or ischemia [4, 5]. The amount of nucleoside formed under such conditions have been suggested to be sufficient to induce coronary vasodilatation [6, 7]. Adenosine is synthesized in vascular endothelium and myocytes [8, 9] and released into surrounding vascular and interstitial compartments during the ischemic and reperfusion periods.

Adenosine is removed from tissues either by phosphorylation to AMP by adenosine kinase or by deamination by adenosine deaminase (ADA). Nees *et al.* [7] suggested that at low concentration, adenosine is phosphorylated (K_m 0.2 μM) while at higher concentration it is actively deaminated (K_m 20–50 μM).

The aim of this study was to analyse adenosine catabolism by adenosine deaminase in plasma, erythrocytes, granulocytes and lymphocytes of patients with stable angina pectoris (SAP).

MATERIALS AND METHODS

Seventy six patients (aged 39–84 years, 39 women and 37 men) with SAP were tested.

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Abbreviations: ADA, adenosine deaminase; SAP, stable angina pectoris

The reference group consisted of 98 clinically healthy subjects (aged 34–87 years, 47 women and 51 men).

In this study venous blood was collected on heparin as anticoagulant from patients soon after the onset of pain (immediately on admission to the hospital).

Erythrocytes and plasma were isolated and lymphocytes and granulocytes were separated in two parallel blood samples. Red blood cells were separated by centrifugation, washed twice with an excess of 0.15 M NaCl and hemolyzed by two freezing and thawing cycles. Adenosine deaminase activity in erythrocytes was determined after Hopkinson *et al.* [10] and expressed in mU/g hemoglobin. One unit of ADA activity was defined as the enzyme activity that catalysed the conversion of 1 μmol of adenosine to urate in 1 min at 37°C ($\varepsilon = 12.5 \times 10^3$ l · mol^{-1} · cm^{-1}).

Adenosine deaminase activity of plasma was determined according to Giusti & Galanti [11] and expressed in U/l. Hemoglobin concentration was determined by the cyanmethemoglobin method with Drabkin's reagent.

Lymphocytes and granulocytes were isolated from peripheral blood by the Ferrante & Thong method [12] modified by Zeman *et al.* [13], employing Gradisol G (Polfa) preparation. Centrifugation of whole blood (5 ml) for 30 min at 400 × *g* over Gradisol G (3 ml, of 1.115 g/ml specific density) at 20–25°C yielded an interphase, the upper part of which contained lymphocytes and the lower one, granulocytes. Isolated lymphocytes and granulocytes were washed in phosphate buffered saline (PBS), pH 7.0, and suspended, after centrifugation in the exactly the same volume of PBS solution. Türk dye solution was added to the samples of these suspensions and the number of cells was counted in Bürker chamber. Granulocytes and lymphocytes were removed from PBS suspension by centrifugation. Granulocytes and lymphocytes were lyzed in the presence of 1% Triton X-100 solution by a single freezing and thawing cycle. Adenosine deaminase activity in lysates of granulocytes and lymphocytes was determined by the method of Hopkinson *et al.* [10] and expressed in mU/10^6 cells.

Student's *t*-test was used to analyse the results.

RESULTS AND DISCUSSION

The activity of adenosine deaminase in granulocytes and lymphocytes of patients with stable angina pectoris was lower by 27% and 24% respectively as compared with control group ($P < 0.001$), whereas the differences in the corresponding activities in plasma and erythrocytes were insignificant although somewhat lower than in the control subjects (Table 1). Our data on ADA activity in plasma of the patients with stable angina pectoris are consistent with those reported by Ungerer *et al.* [14] and Gakis *et al.* [15] in human serum. According to the first group of authors ADA activity in human serum is 15 U/l [13] and according to Gakis *et al.* [15] it

Table 1. Activity of adenosine deaminase (ADA) in granulocytes, lymphocytes, erythrocytes and plasma of patients with stable angina pectoris (SAP)

The data represent means ± S.D. from the number of subjects indicated in parentheses. For analytical details see Materials and Methods.

	ADA activity			
	Control group		SAP patients	
1. Granulocytes, mU/10^6 cells	45.2 ± 14.4	(92)	33.0 ± 13.9***	(66)
2. Lymphocytes, mU/10^6 cells	47.1 ± 12.4	(94)	35.7 ± 11.5***	(69)
3. Erythrocytes, mU/g hemoglobin	834.3 ± 220.0	(98)	836.4 ± 171.1^{NS}	(76)
4. Plasma, U/l	16.5 ± 4.6	(93)	18.3 ± 5.0^{NS}	(48)

***Significant $P < 0.001$; ^{NS} non significant.

is 18 U/l. Russo *et al.* [16] reported that ADA activity in the leukocytes of healthy subjects amounts 28.41 ± 3.93 nmol/mg protein.

The mechanism by which endogenous adenosine protects heart from the ischemia-reperfusion injury may involve different adenosine receptors (A_1, A_2, A_3), linked by a variety of effector mechanisms to the GTP-binding proteins. In humans ADA occurs in different isoenzymatic forms: A_1, A_{1+CP} (A_1 + glycoprotein combining protein) and A_2. In general, in human tissues ADA activity is attributed mainly to ADA_1. Contribution of ADA_2 in total ADA activity is minor. This isoenzyme differs in relative molecular mass, kinetic properties and distribution: ADA_1 is an intracellular isoenzyme while ADA_2 and ADA_{1+CP} are extracellular ones [17]. In human serum and plasma the extracellular forms ADA_2 and ADA_{1+CP} are prevailing [14]. This last isoenzyme was found to be mainly bound to membranes and named an ecto-ADA [18].

The lowered ADA activity in granulocytes and lymphocytes of patients with stable angina pectoris indicating less intensive catabolism of adenosine can not be interpreted univocally. It might suggest that adenosine preserved in these blood cells is directed to ATP synthesis required for activation of polymorphonuclear leukocytes which in turn are trapped in the coronary circulation [19] and are infiltrating the ischemic region [20]. This might induce inflammatory reaction and clear up the irreversibly damaged and necrotic tissue.

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