Increased adenosine deaminase serum activity in patients with acute myocardial infarction

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Abstract

With the aim to evaluate adenosine deaminase serum activity in patients with acute myocardial infarction, a prospective, observational study were done in 56 patients admitted to the central hospital emergency room with a diagnosis of acute myocardial infarction; 32 had elevation of ST segment in the electrocardiogram, of which 24 underwent thrombolysis therapy and 14 evolved a Q wave. Each patient was submitted to a clinical evaluation and serum activity of T troponin, creatine kinase, creatine kinase MB isoform of and adenosine deaminase were determined. Results illustrated that adenosine deaminase serum activity was 109.6 ± 8.34 nmol/mL, decreasing after treatment to 82.34 ±7.45 nmol/mL and 81.82 ± 9.40 nmol/mL at 24 and 72 hours, respectively, which it was significantly related in those patients that displayed no EST in electrocardiogram and received no thrombolytic treatment. In these patients adenosine deaminase activity was correlated with creatine kinase activity. In patients with elevation of ST segment (independently of developing a Q wave) receiving thrombolytic treatment, the adenosine deaminase serum activity remained elevated and unchanged. In conclusion: adenosine deaminase levels can be used as a molecular marker of the evolution in acute myocardial infarction patients receiving no thrombolytic therapy and presenting no changes in the ST segment.

KEY WORDS: Adenosine Deaminase, Acute Coronary Syndrome, Acute Myocardial Infarction, Creatine kinase.

Introduction

Cardiovascular diseases are responsible for 30% of deaths worldwide and 80% in developing countries[1]. In Venezuela, Acute Myocardial Infarction (AMI) ranks first among causes of death from chronic no communicable diseases (21.9%), predominantly in males between 40 and 60 years of age[2].

AMI is a term used to describe acute necrotic changes in the myocardium due to sudden deprivation of coronary blood supply (i.e., acute coronary occlusion or hemorrhage), which causes hypoxia or anoxia, forcing the cardiomyocyte metabolism to change from aerobic to anaerobic[3].

During this process, oxygen free radicals and cellular acidosis are generated[4] and high-energy phosphates are rapidly consumed[3].

Under conditions of tissue damage, endogenous adenosine concentration rapidly raises; furthermore, the activation of G protein-coupled adenosine receptors is responsible for changes such as vasodilatation, inhibition of inflammation, modulation of the sympathetic nervous system activity, and protection against the deleterious consequences of ischemia-reperfusion[5].

Adenosine levels are regulated by the activity of the enzyme adenosine deaminase (ADA), which is a cytosolic enzyme of the purine catabolic pathway. This enzyme catalyzes the deamination of adenosine and 2'-deoxyadenosine to produce inosine and 2'-deoxyinosine respectively; ammonia is produced as a byproduct in this process[6]. The

physiological role of ADA is not entirely clear; however, various studies indicate that ADA activity in plasma of patients with chronic hypoxia is higher as compared with healthy patients, indicating that induction of ADA activity represents a physiological adaptation to high levels of adenosine during hypoxia[7]. Therefore, we propose that as the serum ADA activity is elevated during AMI, its activity correlates to the intensity of cellular hypoxia.

The aim of this study was to evaluate the ADA levels in serum samples of patients with AMI and correlate it to EKG ST segment changes, Q wave, use of thrombolytic therapy, and the serum activity of AMI classic enzyme markers (i.e., CK and CKMB) in order to explore the role of ADA as a biochemical marker for AMI prognosis.

Materials and methods

Population: 56 patients (males and females) older than 18 years, admitted to the University Central Hospital's emergency room (Barquisimeto, Venezuela) between September 2008 and June 2009, with an AMI diagnosis. All patients received a thorough clinical exploration to evaluate the characteristics of the AMI pain (i.e., onset, location, duration, radiation, and possible triggering activity) and concomitant symptoms and signs: heart rate, blood pressure, and a 12-lead electrocardiogram were recorded for all patients. Exclusion criteria were: less than 18 years of age, sepsis, pneumonia, bronchopneumonia, intestinal malabsorption syndrome, asthma and other immune disorders, and signs of dementia and/or psychosis. Patients gave informed consent, which was approved by the Ethics Committee of the Centroccidental University "Lisandro Alvarado", Health Sciences School.

Samples: 2-5 ml blood samples (two per patient) taken from the antecubital vein at 0, 24, and 72 hours of the patient admission were tested for routine hematology parameters as well as for T-troponin and time-dependent determination of the CK, CK-MB, and ADA activity.

ADA levels: 5-7 mL of blood without anticoagulant was allowed to cloth at room temperature (25°C) for at least two hours, centrifuged at 3000 rpm for 30 minutes, and the sera stored in Eppendorf's tubes at -20°C until processing. Before testing, samples were slowly thawed and the ADA levels measured spectrophotometrically using a colorimetric method as described by Giusti and Galanti[8].

Statistical analysis: Results are presented as mean ± standard error (SE). A one-way ANOVA and Dunnet post-test were performed to establish whether significant differences existed between samples. A p value <0.05 was considered statistically significant. Statistical analysis was performed using the Graph Pad Prism 4 software.

Results

The population included 36 male and 20 female, within the range of 31 to 90 years of age (average age of 61.52 ± 1.58 years), all diagnosed with AMI at admission. 10 patients had established history of hypertension and diabetes; 38 patients had a single antecedent: arterial hypertension (n = 33), diabetes mellitus (n = 3), and ischemic heart disease (n = 2); 10 patients had combined antecedents of arterial hypertension and diabetes and 1 had ischemic heart disease and diabetes; 7 patients had no apparent predisposing history. The predominant symptom in all these patients was ischemic chest pain with classical irradiation, being the intensity higher in patients that displayed Q wave; ischemic pain improved after treatment was initiated. AMIs of anterior location were the most frequently (24 for anterior alone and 4 for anterolateral), followed by lateral and inferior (13 for each one); 2 patients exhibited an extensive AMI which extended more than two cardiac walls (for more information see Table I).

Table I. Clinical characteristics of the patient groups

		STSE-Q+	STSE-Q-	No-STSE
Gender	**M**	**10**	**13**	**13**
	F	**3**	**6**	**11**
Age		61,08 ± 2,83	61,79 ± 2,87	61,54 ± 2,56
Personal Antecedents				
Hypertension		13 (100 %)	12 (63,16 %)	18 (75 %)
Diabetes		4 (30,77 %)	4 (21,05 %)	6 (25 %)
Ischemic		0 (0 %)	0 (0 %)	3 (12,5 %)
Pain intensity		7,85 ± 0,48*	6,41 ± 0,62	5,33 ± 0,59
Heart rate		82,69 ± 4,65	86,05 ± 4,06	80,13 ± 2,42
Systolic tension		135,3 ± 8,44	128,3 ± 4,30	133,5 ± 4,88
Diastolic tension		79,85 ± 4,89	81,63 ± 2,67	81,38 ± 2,81
Localization				
Anterior		4 (30,77 %)	14 (73,68 %)	6 (25 %)
Lateral		4 (30,77 %)	1 (5,26 %)	8 (33,33 %)
Anterolateral		0 (0 %)	0 (0 %)	4 (16,66 %)
Inferior		4 (30,77 %)	4 (21,05 %)	5 (20,83 %)
Extensive		1 (7,69 %)	0 (0 %)	1 (4,17 %)

STSE means ST segment elevation; Q the presence (+) or absence (-) of Q wave. The pain intensity was subjectively obtained from the patients in a scale 0 to 10. *means $p < 0.05$ when STSE-Q+ and Non-STSE were compared by Bonferroni's post-test.

The admission EKG demonstrated a ST segment elevation in 32 patients, but only 3 had a concomitant Q wave. Of those patients admitted with ST segment elevation, but no Q wave, 10 developed a Q wave after 24 and 1 patient developed it 72 hours after admission. 24 ST segment elevation positive patients received thrombolytic therapy. The patients with no ST segment elevation and no thrombolytic treatment did not develop a Q-wave in the EKG.

CK, CK-MB, and T-troponin serum activities were elevated in all patients with clinical diagnosis of AMI. CK and CK-MB serum levels decreased significantly and progressively after 72 hours and the lowering of these enzymes' level was related with clinical and EKG improvement (see table II).

Table II. CK, CK-MB and ADA serum activities in ami patients with diferent ekg profiles

	TIME (Hours)	CK (UI/dL)	CKMB (UI/dL)	ADA (nmol/mL)
STSE-Q+	0	546.3 ± 114.5	116.3 ± 33.14	91.62 ± 11.41
	24	612.8 ± 136.3*	119.3 ± 30.67	73.11 ± 9.52
	72	414.7 ± 100.0*	56.39 ± 9.783*	89.39 ± 25.46
STSE-Q-	0	400.5 ± 73.23	88.01 ± 13.78	120 ± 19.64
	24	430.1 ± 106.4*	79.66 ± 18.57	95.43 ± 17.12
	72	286.5 ± 88.52*	40.43 ± 8.112*	101.5 ± 15.04
No-STSE	0	316.4 ± 47.92	76.19 ±13.92	115.4 ± 15.16
	24	272.9 ± 35.47*	60.42 ±11.36	75.23 ± 10.18*
	72	174.6 ± 25.64*	29.44 ±6.153*	51.12 ± 7.36*

STSE means ST segment elevation; Q the presence (+) or absence (-) of Q wave. *means $p < 0.05$ when 24 and 72 hours are compared with 0 hours.

All patients diagnosed with AMI had elevated serum levels of ADA at admission, which also decreased significantly after 24 and 72 hours. The average levels were 109.6 ± 8.34 nmol/mL at 0 hours, 82.34 ± 7.45 nmol/mL at 24 hours and 81.82 ± 9.40 nmol/mL at 72 hours.

When performing an analysis of enzyme activity related to EKG changes, we noticed that the decrease in these enzymes serum activity were statistically significant at 24 and 72 hours in no ST segment elevation patients and in patients that did not received thrombolytic therapy (Figures 1 and 2).

Figure I

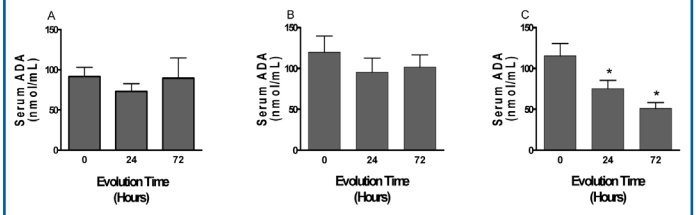

Figure 1. ADA levels in the serum of AMI patients with different EKG profiles. (A) Patients with ST segment elevation and Q wave on EKG record; (B) patients with ST segment elevation and no Q wave; and (C) patients with no ST segment elevation and no Q wave. *$p < 0.05$ for 24 and 72 hours compared to 0 hours.

Figure II

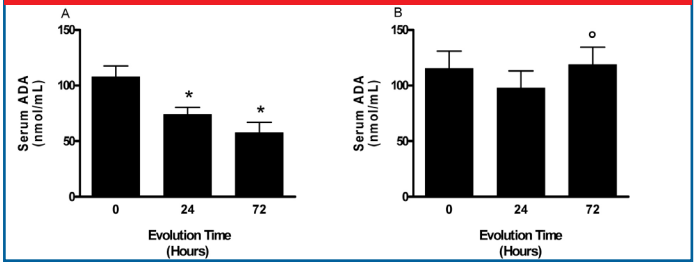

Figure 2. Effect of thrombolytic treatment on the ADA levels in serum of AMI patients. (A) No thrombolytic treatment; (B) thrombolytic treatment. *$p < 0,05$ for 24 and 72 hours compared to 0 hours; °$p < 0.05$ for ADA levels at 72 hours for both groups.

ADA activity was positively correlated with the CK activity in patients with no ST segment elevation and in patients that did not receive thrombolytic treatment. On the other hand, in patients with a ST segment elevation that evolved to Q-wave, no significant correlation was established between ADA and CK (Figure 3 and Table 3). CK-MB and ADA serum activities were correlated with the sera CK and ammonia levels, respectively, for all groups of the patients, validating the lab methodology.

Table III. Correlation of ADA, CK, and CKMB serum activities in ami patients with different ekg profiles

		r^2	Pearson's r	p
CK vs CKMB	STSE-Q+	0.15	0.39	0.01
	STSE-Q-	0.16	0.40	0.04
	No-STSE	0.15	0.38	0.001
ADA vs CK	STSE-Q+	0.006	-0.023	0.89
	STSE-Q-	0.011	-0.1	0.49
	No-STSE	0.11	0.32	0.01
ADA vs CKMB	STSE-Q+	0.003	-0.057	0.75
	STSE-Q-	0.017	0.13	0.38
	No-STSE	0.001	0.033	0.8

STSE means ST segment elevation; Q the presence (+) or absence (-) of Q wave.

Figure III

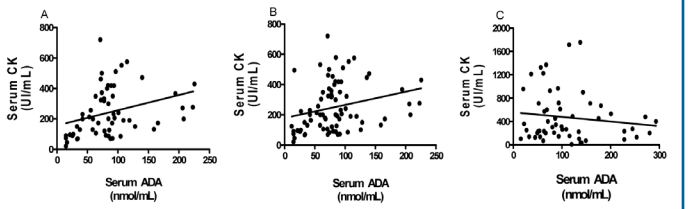

Figure 3. Correlation between ADA and CK serum levels in AMI patients. (A) patients with no ST segment elevation and no Q wave (r2 = 0.11; Pearson's r = 0.32; p = 0.01). (B) AMI patients receiving no thrombolytic treatment (r2 = 0.07; Pearson's r = 0.26; p = 0.02). (C) AMI patients receiving thrombolytic treatment (r2 = 0.02; Pearson's r = -0.13; p = 0.34).

In this study, we found no significant correlations among the ADA and/or CK-MB serum levels and the patients' sex and/or age.

Discussion

In this paper we have presented data demonstrating that ADA serum activity was elevated in patients with an AMI diagnosis at the time of hospital admission; which decreased after 24 and 72 hours of treatment onset. The diminished ADA activity was correlated to the absence of thrombolytic treatment in patients with no ST segment elevation in the EKG; ADA serum activity was positively correlated with serum CK activity.

It has been demonstrated that hypoxic events induce an elevation of endogenous adenosine in synaptic and intercellular spaces[9], which reflects a high rate of ATP utilization and depressed aerobic ATP production as a result of hypoxia, leading to elevated levels of AMP, which is converted to adenosine by the action of the 5'-nucleotidase enzyme[10]. The elevation in the level of ADA in serum of

ischemic patients suggests an adaptive metabolic phenomenon where high levels of adenosine are produced during tissue hypoxia.

Adenosine is a nucleoside that counteracts the deleterious effects of ischemia as it promotes coronary vasodilatation through the activation of A2 receptors[11] and decreasing the oxygen consumption through activation of A1 receptors[12]. Also, adenosine is able to attenuate cell damage induced by reperfusion[13], an effect attributed to mechanisms related to the preservation of ATP, since adenosine stimulates glycolysis by increasing glucose uptake[14], inhibits the release of norepinephrine acting on A2 presynaptic receptors[15,16], and counteracts adrenergic effects via A1 receptors[17]. Furthermore, this nucleoside inhibits the activation and adhesion of neutrophils trough A1 and A2 receptors, respectively[18-20] and inhibits platelet aggregation by acting on A2 receptors[21,22]; both effects improve coronary circulation and counteract ischemia and reperfusion damage by inhibiting the production of free radicals.

Elevation of ADA during hypoxic events has been reported by Eltzschig et al., (2006)[7] in pediatric patients with chronic hypoxia product of a heart congenital disease. The authors, utilizing microarray technology, demonstrated that the endothelial ADA gene expression is induced by hypoxia. Similarly, Kaul et al., (2006)[23] reported that the ADA activity and serum malondialdehyde (MDA) were elevated in patients with AMI undergoing thrombolytic therapy, suggesting that the elevation of ADA is associated with reperfusion injury in these patients. These results are consistent with our data, we observed that serum ADA levels remained elevated after 24 and 72 hours in patients receiving thrombolytic therapy, while decreasing significantly in patients that did not receive such therapy. The decreased ADA levels in no ST segment elevation patients' sera suggest that hypoxic events were completely reversed, with no permanent tissue lesion or necrosis.

ADA can usually be found attached to the endothelial cell membrane interacting with CD26 protein[7]; the ADA-CD26 interaction can be disrupted by thrombolytic enzymes, releasing ADA into the blood and thus increasing its serum levels in those patients receiving thrombolytic treatment.

The correlation between the serum ADA and CK levels in no ST segment elevation patients not received thrombolytic treatment, indicates that the elevation of ADA is not a phenomenon restricted to heart tissue, but reflects a wider occurrence involving other tissues, especially those that would be affected by cardiac dysfunction. In this way, it has been reported that serum levels of adenosine and CK are elevated in patients who had pulmonary edema[24]; taking together these and our data, we propose that the elevation of ADA reflects a phenomenon involving pulmonary hypoxia associated to ventricular dysfunction.

Finally, our findings suggest that in those patients diagnosed with AMI, their serum's ADA level became elevated as consequence of myocardial and/or pulmonary hypoxia. The level of this enzyme decreased after 24 and 72 hours of the supportive treatment onset in no ST segment elevation patients that did not receive thrombolytic therapy. Because AMI patients with EKG signs of myocardium lesion and/or necrosis that received thrombolytic treatment maintained high levels of ADA, this enzyme could be considered a prognostic marker only in those patients with no ST segment elevation EKG and receiving no thrombolytic therapy. The elevation of serum ADA activity in patients receiving thrombolytic treatment could reflect the ADA release from its endothelium attachment by the action of thrombolytic enzymes.

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