Res Exp Med (Berl) (1982) 181: 181–187

Research in
Experimental Medicine

The Prognostic Value of Serum Acetylcholinesterase in Myocardial Infarction

Theoretical and Clinical Considerations

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Summary. The aim of the present work was to determine experimentally the prognostic value of reduced activity of serum acetylcholinesterase (ACE) in cases of acute myocardial infarction (AMI). In the first part of the research we studied the mechanism of this reduction by comparing the serum results with the levels of the enzyme in cardiac tissue in such cases. It was found that ACE activity is reduced in serum and also in cardiac tissue in AMI, in contrast to creatine kinase (CK) that is augmented in serum but reduced in cardiac tissue. This fact was interpreted as a "reduced cardiac flow of ACE," in contrast to the "augmented cardiac clearance of CK" in similar cases.

In the second part of our research, 50 patients, admitted in our hospital for AMI, were examined and their blood analysed mainly for ACE and CK at brief intervals (every 2–3 days), during hospitalization, and thereafter every 1–2 weeks for 1–4 months. From the results of ACE activity it was possible to classify these variations into four groups, each of them having a defined prognostic value for the evolution of the AMI, a persistent reduced serum activity being interpreted as a bad, severe prognosis, with high morbidity or mortality (groups II and III). We suggest, therefore, that the determination of ACE in serum in cases of AMI, especially before discharge home of such patients, may be an additional useful laboratory test in such cases.

Key words: Serum acetylcholinesterase – Creatine kinase – Myocardial infarction

Introduction

Moore et al. [5] have found that serum acetylcholinesterase (ACE) is reduced in acute myocardial infarction (AMI), in contrast to the increased activity of serum creatine kinase (CK). The mechanism and the clinical importance of the increased CK level in AMI were studied extensively during the last two decades

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0300-9130/82/0181/0181/$ 1.40

[2, 4, 6, 11], for the diagnosis and the determination of the infarct size, but no report has been published since 1957 on the importance of the reduced ACE for the diagnosis and/or prognosis of the AMI.

The first part of our report examines the variations of ACE in serum and in cardiac tissue in such cases. The second part deals with the clinical application of these variations.

Material and Methods

Our material for analysis comprised: ten post mortem specimens from infarcted cardiac tissue and ten sera of the same patients suffering from AMI previously examined for ACE and CK. Ten sera from normals and four post mortem heart biopsies from individuals not suffering from AMI served as controls. All cardiac specimens were taken not more than 12 h after death.

The fresh cardiac specimen was immediately homogenized in a Potter-Helvenjem homogenizer by addition of a cold phosphate-natrium chloride (1/l) buffer, 0.01 *M*, pH 6.5, added in three subsequent portions, and rapidly frozen and thawed after each addition for better homogenisation. Finally, it was diluted with the buffer to 1/10 (g/tissue vs. ml of buffer) and centrifuged in the cold at 1,500 × *g* for 5 min. CK and ACE were determined on these extracts and in sera: CK by the method of Menache and Gaist [4], ACE by the method of Nathelson [6]. CK-MB fractions were determined by the method of the Helena Laboratories, by electrophoretic separation and fluorimetric determination (Procedure no. 20, 5/1981).

Fifty patients (30 males and 20 females) aged between 37 and 72 years (mean 55 years), admitted in our hospital for AMI, were examined. The mean duration of hospitalisation was 16 days (range 10–57 days). The criteria for the diagnosis of AMI were the known clinical typical symptoms: ECG changes, and elevation of serum CK at least. All patients were monitorised for a minimum of 48 h.

Occasional premature beats were recorded in most patients. The following complications were also noticed in approximately 30% of cases: multiple ventricular premature beats (two patients), atrioventricular block of various degrees (three patients), paroxysmal atrial fibrillation (four cases) left ventricular failure (two cases), pulmonary embolism (two patients), and embolism of the left femural artery (one patient). A pacemaker was inserted in one patient with atrioventricular block. Two patients with ventricular fibrillation were successfully resuscitated, and one of them had pericarditis later on. Five patients suffered from recurrent MI during hospitalisation, and one after release home. Six patients died, one during hospitalisation and five thereafter, at different intervals (one after 90 days).

The following enzymes were investigated in the sera of these patients until release from the hospital: aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), CK, CK-MB fraction and ACE. AST and ALT were determined as described by Henry et al. [2], and LDH according to Kachmar et al. [3]. CK, CK-MB fraction and ACE were determined as mentioned before.

All patients available were re-examined clinically and their blood was reanalysed several times at the hospital and after their release home, for further 1–4 months.

Results

The results obtained for the CK and ACE in normal and in infarcted tissue, in normal sera and in sera from AMI patients are shown in Table 1. Figure 1 shows two examples of isoenzyme patterns of CK in normal (a, b) and infarcted (c, d) cardiac tissue. Comparing the results of ACE between infarcted cardiac tissue and serum from AMI, a positive correlation in the direction of a concomitant reduction in both activities is found (Table 1). In contrast, a negative correlation

Table 1. CK and ACE values in normal and infarcted cardiac tissues and in sera from normal and infarcted individuals

Materials	No. of cases	ACE	CK (I.U./g wet tissue)	
			Total	MB fraction
Normal cardiac tissue	4	935 ± 80	9,600 ± 1,000	3,360 ± 800 (35%)
Infarcted cardiac tissue	10	635 ± 50	1,475 ± 400	300 ± 100 (20%)
Normal serum	10	240 ± 55	40 ± 10	2%
Serum of AMI patients	10	150 ± 30	150 ± 50	8%

The coefficients of correlation between cardiac tissue and serum and the levels of significance in AMI were as follows:

$$\frac{\text{CK in cardiac tissue}}{\text{CK in serum}} = -0.85 \qquad P = 0.03$$

$$\frac{\text{ACE in cardiac tissue}}{\text{ACE in serum}} = +0.75 \qquad P = 0.02$$

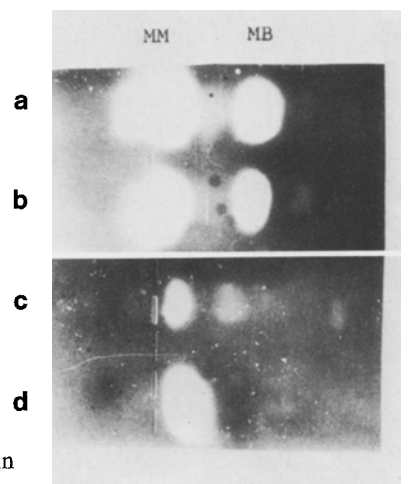

Fig. 1. Two examples of isoenzyme pattern of CK in normal (**a, b**) and in infarcted cardiac tissue (**c, d**)

may be observed for CK (total and MB fraction), i.e. a diminished activity in infarcted cardiac tissue and an elevated activity in serum from AMI (Table 1).

The findings for total and CK-MB activities are known in the literature as an "increased cardiac clearance" or as a "cardiac depletion" [9] of the enzyme in AMI. This fact was studied widely for the determination of the infarct size and its treatment [7, 8, 10], although some restrictions are expressed in more recent papers [1].

Our findings for ACE may be defined as a "reduced cardiac flow" of the enzyme in AMI as primary cause or as secondary effect, since the blood flow to ischemic regions (as are the infarcted cardiac areas) are severely affected. In the

first part of the discussion we try to give an explanation or interpretation of these results.

From the observed changes of ACE activity in serum during the follow-up of the 50 patients with AMI, when hospitalised and after their release home, we may classify these variations into four different groups as follows (Table 2):

Group I

In 16 of the 50 patients (32%) the enzymatic changes had the following pattern: the initially normal value of ACE activity was followed by a decline and later on (after 7–11 days) returned to normal values. AST, CK and LDH showed high initial levels followed by a gradual decline to normal values. The clinical course of these 16 patients was uncomplicated, and they were released home in a good condition, returning later on to their routine and regular work life.

Group II

A second pattern of enzymatic changes for ACE activity was seen in 14 patients (28%). After the first decline in ACE levels, the enzyme returned very slowly to the normal values 15–30 or more days later, and usually after the patient left the hospital. The changes of AST, CK and LDH, however, were similar to those of the first group. In addition, nine of these 14 patients (66%) had various complications. One had ventricular fibrillation on day 2 and was resuscitated, two developed congestive heart failure, and one had paroxysmal atrial fibrillation. Three patients (21%) of this group died, one on day 13, another 5 days after being released from the hospital, and a third 20 days after release.

Group III

Eleven of the 50 patients (22%) showed an extension of the AMI and a third pattern of enzymatic changes expressed by typical ACE alterations, two declines, and also changes of the other enzymes, especially CK, two elevations.

One patient had AMI with initial enzymatic changes as in group II. After 1 week she had an extension of the infarction which was confirmed by ECG changes and a second increase in CK, AST and LDH levels. At the same time, ACE activity showed a second decline and remained low despite the return to normal values of the other enzymes (except a slight elevation of LDH), until the patient was released from the hospital, suffering from congestive heart failure.

Two patients had also an extension of the infarction. However, ACE returned to normal values rapidly after the second nadir, and these patients were released in good condition. A fourth patient suffered from pulmonary embolism documented also by increased LDH and AST and a second decline in ACE. The enzymatic changes returned to normal values later on, when the patient left the hospital. Another patient with ventricular fibrillation suffered subsequently from pericarditis, pneumonia and urosepsis, and he was released home after 32 days with congestive heart failure and low ACE activity. Both patients improved later as found after 6 weeks upon re-examination.

Table 2. Evolution of AMI and variations in serum ACE in the 50 patients during the 1–120-day follow-up

Group no.	ACE variations	Clinical course	Haemodynamic data	Prognosis
I. 16 patients (32%)	First decline of the enzyme and return to normal value within 7–11 days	Uncomplicated. Patients released home in good condition	–	Generally good
II. 14 patients (28%)	First decline of the enzyme and return to normal value *very slowly* 15–30 days, often after release home	Nine of the patients (66%) had various complications	Eighteen patients of the two groups, II and III, presented a positive significant correlation ($P = 0.01$) between metabolic acidosis + hypokalemia and low ACE values	Three patients (21%) released home with low ACE value (50% of the normal) died thereafter at different intervals
III. 11 patients (22%)	One or two declines of the enzyme with *persistent very low values* (below 50% of the normal)	Six patients of this group (55%) presented an extension of the MI and various serious complications		Three patients (27%) released with ACE values below 50% of the normal died later at home
IV. 9 patients (18%)	No clear correlation between all the enzymes values (CK, LDH, GOT and ACE) and diagnosis or prognosis			

Three patients (27%) of this third group, released from the hospital in good condition but with very low ACE activity (below 50% of the normal value), died afterwards: one on day 30, another after 50 days, and a third after 3 months from their release home.

Group IV

A fourth pattern of enzymatic changes was found in nine patients (18%). No clear correlation was found in these patients between all enzymatic changes, on the one side, and the diagnosis or prognosis of the AMI, on the other. In two patients the initial ACE activity was lower than the subsequent activities, but CK and the other enzymes showed no clear changes during hospitalisation.

A 42-year-old man of this group died on day 8 of hospitalisation with very low and progressively decreasing ACE activity and with no clear variations in the other enzymes studied, except for a high level of LDH values.

Discussion

With the aim of explaining the reduced ACE in infarcted cardiac tissue, we have investigated the presence of an inhibitor of the enzyme in this tissue. An inhibition of activity of ACE was found with minimal additions of extract of infarcted tissue to extract of normal tissue or to normal serum, causing an accumulation of intact substrat in the incubation mixture in vitro. Due to the cellular muscle membrane lesion caused by the AMI, this inhibitor may be cleaved also in peripheral blood in vivo and may cause a reduction of ACE activity in serum. These findings are in accordance with the results of Bettini et al. [1] who found a contraction of cultured coronary segments by addition of acetylcholine HCl substrat to the cultured segments.

The findings of our study have a practical appliance. In the clinical follow-up of the 50 patients hospitalised for AMI, a negative correlation between the levels of CK, AST, LDH, on the one hand, and ACE activity, on the other, was found in 40 of them (80%), in the first days of hospitalisation. All the patients in whom ACE returned to normal values within 5–10 days had a good prognosis (group I of ACE pattern). In contrast, congestive heart failure upon release of the patient from the hospital was invariably associated with low ACE activity (group II of ACE pattern). Furthermore, 23 of the 50 patients (46%) with final low ACE had one or more complications during the hospitalisation or subsequently (groups II and III). Moreover, six of the latter died, having a very low ACE activity (less than 50% of the normal) before they died, even though they had normal values in other enzymatic tests and no other measurable clinical complications.

The results of serum ACE were compared with the haemodynamic data in blood samples taken simultaneously for this purpose. It was found that 80% of the patients in groups II and III presented a non-respiratory metabolic acidosis, often as a consequence of hypoxia. In most cases this state was associated or followed by an electrolytic imbalance, reduced serum kalium concentration mainly, and persistent modifications of the T-wave of the echographic tracement. A positive

correlation ($P=0.01$) between hypokalemia and reduction of serum ACE activity was found in such patients, in 60% of the cases where the serum K was below 3.0 mEq/l and the ACE below 70% of the minimal normal value. The correction of the K value and the acidosis by convenient treatment have not modified the reduced ACE value, associated with the morbidity and/or the mortality risk, a fact that supports our view of the prognostic importance of ACE in such cases.

Thus, it appears that a persistent low ACE activity in patients with AMI is generally accompanied with a high morbidity rate and poor outcome for the patients. We conclude, therefore, that the determination of serum ACE activity may be a useful laboratory test of prognostic value in patients hospitalised for AMI, especially before their release home, in addition to the complex physical medical examination and the instrumentation available today in a modern cardiac care unit.

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Received July 16, 1981 / Accepted July 26, 1982