Serum peptidases in myocardial infarction

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The activities of three peptidases, glycyl peptidase, γ -glutamyl transpeptidase, and leucine aminopeptidase, were determined at regular intervals in the sera of 51 patients with myocardial infarction. The activity of glycyl peptidase rose significantly on the second or third day after infarction, while significant increase in γ -glutamyl transpeptidase and leucine aminopeptidase occurred by the end of the first and second weeks, respectively. All three peptidases studied reached top values at the beginning of the third week and slowly decreased thereafter. Six weeks after myocardial infarction, however, their activities were still significantly increased. Starch gel electrophoretic studies performed at the time of peptidase maximal rise revealed two serum fractions with γ -glutamyl transpeptidase activity: one occupied the region between fast α_2 -globulin and the β -globulins, the other migrated with the β -lipoproteins. The increase in total leucine aminopeptidase activity was accompanied by intensification of the enzymatic fraction occupying the zone between the post-albumins and fast α_2 -globulin, and by the appearance of a new fraction located in the sector between fast α_2 -globulin and β -globulins. Though this pattern of distribution of both peptidases was a consistent finding after myocardial infarction, an identical pattern was observed in some cases of hepato-biliary diseases. Clinical observations indicate that determination of serum peptidase activities, particularly that of γ -glutamyl transpeptidase, may be a useful late enzymatic test for myocardial infarction.

It has been noticed in our department that the serum γ -glutamyl transpeptidase (GGTP) activity rises in some patients after myocardial infarction (Orłowski, 1963). Agostini, Ideo, and Stabilini (1965) showed that the serum enzyme activity was normal during the first days after infarction, reached maximum levels in the second week, and did not return to normal range until after 30 days. These observations received firm support from the results published by other authors (Hedworth-Whitty, Whitfield, and Richardson, 1967; Dimov, 1968; Szczeklik *et al.*, 1968; Ravens *et al.*, 1969; Kędra, and Kolber-Postępska, 1970). Since typical changes in this activity were found in the majority of patients with myocardial infarction, it has been suggested that determination of serum GGTP activity might be of diagnostic value as a late enzymatic test for myocardial infarction (Szczeklik *et al.*, 1968).

Contrary to GGTP, little attention has been given to the behaviour of other serum peptidases in myocardial infarction. Both nor-

mal and raised levels of serum leucine aminopeptidase (LAP) activity were recorded after infarction (Vokurková and Továrek, 1963). Agostini *et al.* (1965) stated that the serum LAP values were within normal range in their patients, but did not specify at what stage of myocardial infarction the determinations were performed. Dimov (1968), on the other hand, found a positive correlation between the serum GGTP and LAP levels in myocardial infarction. Scanty and discrepant results on the behaviour of serum LAP in myocardial infarction, as well as lack of information on other peptidases, led us to study this subject. The present paper reports the behaviour of three serum peptidases, namely glycyl peptidase (GP), γ -glutamyl transpeptidase (GGTP), and leucine aminopeptidase (LAP) in myocardial infarction. Results of studies on the heterogeneity of serum peptidases in myocardial infarction are also presented.

Material and methods

Fifty-one consecutive patients with myocardial infarction (39 men and 12 women) ranging in age

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TABLE Activity of serum peptidases in 38 patients with myocardial infarction

Group	GP activity (IU)		GGTP activity (IU)		LAP activity (IU)	
	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value
Healthy subjects	23.0 $\pm$ 5.7		3.1 $\pm$ 1.6		28.2 $\pm$ 6.4	
Myocardial infarction						
1-3 days	37.2 $\pm$ 14.4	<0.01	6.8 $\pm$ 2.9	N.S.	27.1 $\pm$ 3.8	N.S.
4-7 days	37.0 $\pm$ 9.5	<0.01	13.7 $\pm$ 5.5	<0.01	30.1 $\pm$ 7.9	N.S.
Second week	49.6 $\pm$ 18.7	<0.01	23.1 $\pm$ 17.0	<0.01	38.0 $\pm$ 12.1	<0.05
Third week	50.6 $\pm$ 20.6	<0.01	24.7 $\pm$ 21.9	<0.01	44.0 $\pm$ 9.2	<0.05
Fourth week	42.4 $\pm$ 15.4	<0.01	20.0 $\pm$ 15.4	<0.01	38.4 $\pm$ 10.2	<0.05
Fifth week	42.2 $\pm$ 12.5	<0.01	16.9 $\pm$ 11.6	<0.01	39.8 $\pm$ 11.8	<0.05
Sixth week	37.7 $\pm$ 10.7	<0.01	13.8 $\pm$ 9.0	<0.01	36.3 $\pm$ 9.0	<0.05

Probability (P value) based on null hypothesis for differences between groups of patients and group of healthy controls computed by Student's 't' test.

NS = not significant ($P > 0.05$).

from 32 to 76 years were studied. The diagnosis was based on the presence of typical pain, serial electrocardiographic changes, and a characteristic rise in serum lactic dehydrogenase (LDH) and aspartate aminotransferase (GOT). Eight patients died in the first week of illness, and 5 others left the hospital before the study was terminated. The subsequent statistical analysis is limited to those 38 patients who survived the period of 6 weeks after coronary occlusion.

Fourteen patients were regarded as having sustained a large infarction on the basis of one or more of the following signs: GOT of over 150 units, LDH of over 1500 units, the development of heart failure, the presence of shock or major dysrhythmia. Subendocardial infarction was diagnosed in 7 patients. The criteria for diagnosis of shock were the following: hypotension with a systolic pressure of 90 mmHg or less as determined by sphygmomanometry, pallor, cold extremities, sweating, and oliguria.

A smaller group of 12 patients admitted because of repeated attacks of cardiac pain, but without subsequent confirmation of certain infarction (the acute coronary insufficiency group), was also investigated. None of these patients showed the characteristic alterations in serial electrocardiographic tracings, a rise in the leucocyte count and erythrocyte sedimentation rate, or typical rise in LDH or GOT levels.

The serum activities of (GP), γ -glutamyl (GGTP), and LAP were determined in all the patients studied. The determinations were carried out on the first or second day of infarction and repeated every other day during the first week of illness, and at least once a week subsequently till the end of the sixth week. LAP activity was determined according to the method of Green *et al.* (1955) with some modifications suggested by Goldburg and Rutenburg (1958). GGTP activity was determined according to the method of Orłowski (1965), using γ -L-glutamyl-p-nitroanilide as substrate. Glycyl- γ -L-glutamyl- β -naphthylamide served as a substrate in glycyl peptidase assay, which was performed as described previ-

ously (Szewczuk, Mulczyk, and Szczeklik, 1969). The activities of the peptidases were expressed in international units (IU) as the number of μ moles of β -naphthylamine or p-nitroaniline liberated in one minute in the assay conditions and were calculated per 1000 ml serum. The normal values obtained in a large group of healthy subjects were the following: GP 15.0-35.0 IU, mean 23.0 IU; GGTP 1.5-8.0 IU, mean 3.1 IU; LAP 19.5-39.5 IU, mean 28.2 IU.

The heterogeneity of the peptidases was studied by means of starch gel electrophoresis. After the electrophoretic separation of serum the active enzymatic fractions were visualized using histochemical techniques. GGTP fractions were localized by the method described previously (Orłowski and Szczeklik, 1967), while fractions showing LAP activity were demonstrated by the method of Dubbs, Vivonia, and Hilburn (1961). The sera of 20 patients were investigated. In the majority, electrophoretic separation was repeated several times at various stages of the disease.

Results

The measurements of serum GP, GGTP, and LAP activities in patients with myocardial infarction are summarized in the Table. All three peptidases showed a frequent increase in activities after myocardial infarction. Mean serum GP levels were already significantly increased on the second or third day after infarction. Subsequently they rose steadily and reached maximal values in the third week. Between the fourth and sixth week the enzyme activity slowly decreased, but did not return to normal values.

In the majority of patients normal values of serum GGTP activity were recorded during the first 3 days after myocardial infarction. Out of 51 patients studied, only in 10 was an increase of activity found in the early period of the disease. This group included 4 patients

with congestive heart failure, 3 patients with chronic cholecystitis or liver cirrhosis, and 3 patients in whom severe precordial pain had preceded for a few days admission to the hospital, thus making the exact time of coronary occlusion difficult to ascertain. After the first 3 to 4 days GGTP activity increased progressively till it reached peak values between the end of the second and the beginning of the third week. These high levels gradually fell over the following month, though 6 weeks after infarction the mean activity was still significantly higher than in normal subjects.

Serum LAP activity was within normal range in the first week after infarction. Only 3 patients with congestive heart failure had increased levels of the activity at that time. In the second week the activity of the enzyme was already significantly increased. It reached peak values in the third week and gradually decreased thereafter.

The frequency of increase in activity was not the same for all the three peptidases studied. A typical course of GGTP activity, with peak values significantly increased in the second and third week of infarction, was observed in 31 out of 38 patients (82%). The similar data for GP and LAP were 28 patients (74%) and 24 patients (64%) respectively. In 5 patients (13%) all the three peptidases remained within normal range during the 6-week observation period. In 5 other patients, an increase in only one of the 3 peptidases was noted (GGTP in 4 patients, LAP in 1).

Six patients with myocardial infarction and shock on admission survived the six-week period and were included in the group subjected to statistical analysis. In 3 of them a typical rise in all 3 peptidases was noted, in 1 only GGTP increased significantly, while in the remaining 2 patients the activity of the peptidases remained unchanged. Comparison of patients regarded as having sustained a large myocardial infarction with those suffering from subendocardial necrosis revealed no significant differences in the course of activity of the peptidases. No significant correlations were observed between the peak peptidase activities and the leucocyte count or serum activities of GOT or LDH. Despite a careful search, there was no evidence that the increased activity of serum peptidases in patients with acute myocardial infarction was associated with the administration of any specific drugs.

The increase in GGTP activity was a frequent finding in patients with acute coronary insufficiency in whom myocardial infarction had been ruled out during clinical observation. Out of 12 patients studied 9 showed an

FIG. 1 In this 49-year-old man, acute myocardial infarction occurred on the 14th day of hospitalization. During the 2 weeks preceding coronary occlusion, he experienced repeated attacks of severe chest pain, but LDH and GOT were within normal range, and the electrocardiogram revealed only transient ST changes. At that time the activity of GGTP had already begun to rise, while that of GP and LAP remained unchanged. After infarction a further increase in GGTP activity occurred and the course of the GP and LAP activities was typical.

obvious increase of enzymatic activity in the second and third week after admission, the mean activities differing significantly from the normal values. The peak values reached by the enzyme in some of these patients were in the same range as those recorded in patients with myocardial infarction. Severe coronary insufficiency preceding for a few weeks acute coronary occlusion was also accompanied by a progressive increase in GGTP activity, which continued to rise after the infarction had occurred (Fig. 1). Contrary to GGTP, the activity of other peptidases was usually within normal limits in patients with acute coronary insufficiency. A rise in GP activity in this group was recorded in 2 out of 12 patients, and in that of LAP in 1.

Separation of peptidases in starch gel electrophoresis After starch gel electrophoresis of normal serum, LAP appeared as a single component located in the sector between fast α_2 -globulin and post-albumin. This single component was also present in sera obtained from patients one week after infarction.

tion when total LAP activity remained within normal range. Increase in total activity in the second and third weeks after infarction was accompanied by intensification of staining of the fast α_2 -globulin zone to post-albumin zone and by the appearance of a new band located in the sector between fast α_2 -globulin to β -globulin. In some patients with much increased activity, an additional band was observed between the origin and α_2 -macroglobulin. We have noticed, however, the presence of the same additional two bands with LAP activity in some patients with hepatobiliary diseases (Fig. 2).

The visualization of zones of GGTP activity in sera with normal enzyme activity was difficult, and very faint zones could only occasionally be detected. Within a few days after myocardial infarction a distinct zone of activity appeared. It occupied the region between fast α_2 -globulin and the β -globulins. Its presence was a consistent finding in all patients who exhibited an increase in total GGTP activity by the end of the first week after infarction. In the second and third weeks of the disease, a further rise of total GGTP activity was followed by the appearance of a supplementary enzymatic zone which migrated with the β -lipoproteins (Fig. 3).

Discussion

The results obtained indicate that the serum activity of all the three peptidases studied, i.e. namely GP, GGTP, and LAP, rises after myocardial infarction. In patients without congestive heart failure or concomitant hepatobiliary disease, the GP activity has already significantly increased on the second or third day after infarction. The levels of the other two peptidases remain unchanged at this time. A sharp rise in the GGTP activity usually occurs between the 4th and 7th days after myocardial infarction, while significant changes in the mean LAP activity can be detected only by the end of the second week. The further course of the mean serum activities is fairly similar. They all reach the top levels at the beginning of the third week and slowly decrease thereafter. However, six weeks after infarction the mean activities of the three peptidases are still significantly raised.

Deviations from this typical course were observed in patients who on admission to the hospital presented with clinical signs of liver congestion due to heart failure or with concomitant cholecystitis or chronic hepatitis. The activities of all the three peptidases were increased in these patients even on the first

FIG. 2 Separation of serum LAP activity by means of starch gel electrophoresis in a patient with myocardial infarction of the inferior wall. Serum obtained on the 12th (A), and on the 24th (B) days after infarction. For comparison, separation of the activity in a patient with acute cholecystitis is shown (C).

day of infarction. Cholecystitis or hepatitis did not seem to affect the further behaviour of the serum peptidases, which continued to rise, reached top values by the second or third week, and then slowly decreased. However, in some cases with early liver congestion the course of the peptidase activities was different. Rapid digitalization leading to re-

FIG. 3 Starch gel electrophoretic separation of GGTP activity. (A) Myocardial infarction (6th day of illness). (B) Liver congestion in a patient with valvular heart disease.

gression of the clinical symptoms of liver congestion was accompanied by a fall in the peptidases. Thus, in 2 out of 4 patients studied, the level of the peptidases was still significantly increased between the 2nd and 5th weeks, but the values recorded at this time were lower than in the first days of infarction. In 2 other patients the enzyme values declined temporarily after clinical improvement of the heart performance and rose 2 weeks later.

The origin of the increased serum activity of GGTP and other peptidases in patients with myocardial infarction is not clear. Two organs, namely the liver and the heart, should be considered as possible sources of the raised serum peptidases in this disease. After the kidney and jejunal epithelium the liver and bile ducts are the tissues with the highest amounts of GGTP and other peptidases (Orłowski, 1963; Naftalin *et al.*, 1969). On the contrary, the human myocardium has only traces of peptidase activities. The serum level of GGTP rises significantly in many hepatobiliary diseases: this has proved to be a valuable aid in the diagnosis of these pathological conditions (Szczeklik, Orłowski, and Szewczuk, 1961; Gibinski, Szaton, and Maraszek, 1963). It may therefore be assumed that the disturbances in hepatic circulation occurring in myocardial infarction give rise to the activity of serum peptidases (Naftalin *et al.*, 1969). Clinical observations, however, give little support for this hypothesis. Congestive right heart failure is not a common finding in myocardial infarction. In our material it was noted in 5 out of 51 patients. It occurred in the early period of infarction, and not in the second or third week when serum peptidases usually reached the top values. Furthermore, the activity of other enzymes considered to reflect liver function, e.g. alkaline phosphatase, phosphohexoisomerase, and aldolase, were within normal range in several patients studied at the time of the maximal peptidase rise.

The present data do not permit the determination of the exact origin of the raised serum peptidases after infarction. We believe, however, that changes occurring in the heart muscle rather than in the liver should be considered as the cause of the phenomena under discussion (Szczeklik *et al.*, 1968). Thus, Ravens *et al.* (1969) demonstrated significant alterations in the activity of free and particle-bound GGTP in the homogenates of canine myocardium within the first days after myocardial infarction. These authors found that 10 days after coronary occlusion in the dog, the activity of GGTP had increased tenfold

in the necrotic area compared with normal tissue. The subsequent late increase of serum peptidases, which occurs within a few weeks after infarction, might be attributed to reparative processes taking place in the heart muscle. Clinical observations and histochemical studies seem to support this hypothesis. The results obtained as well as those reported by other authors indicate that the maximal activity of serum peptidases is observed in the second and third weeks of illness, when reparative processes in the myocardium reach the maximal intensity. By histochemical techniques LAP activity, absent in normal human myocardium, was shown in the healing myocardium of man after myocardial infarction. Monis and Weinberg (1964) found significant activity of this enzyme in connective tissue cells, macrophages and fibroblasts proliferating in the necrotic area of the human myocardium a few weeks after infarction. With progressive fibrosis and scarring, diminution in the aminopeptidase activity paralleled the decreasing cellularity of the lesion. The studies of Albert *et al.* (1966), who demonstrated a high GGTP activity in capillary endothelium, lead us to suppose that the capillaries proliferating in the necrotic area may be an additional source of the peptidases in the healing human myocardium.

Clinical observations indicate that determination of the serum peptidases may be of value in confirming myocardial infarction. Of the peptidases studied, GGTP has proved to be most reliable in this respect. Its maximal activity was on the average eightfold higher than the mean activity in normal subjects, while GP and LAP only doubled their values. Furthermore, the typical late increase of GGTP was observed in over 80 per cent of patients with myocardial infarction, while the frequency of GP and LAP increase was much lower. In our patients we have not observed any relation between the rate of increase of serum peptidase activities and the size of the infarction or its site. Other authors have reported similar observations concerning serum GGTP in myocardial infarction (Hedworth-Whitty *et al.*, 1967). According to our experience, the course of serum peptidase activities seems to be of no prognostic value, since no distinct differences were noticed between the survivors of myocardial infarction and those patients who died.

Diseases other than myocardial infarction are also accompanied by a raised activity of the peptidases in the serum. In the overwhelming majority of patients, alterations in the serum peptidases reflect disorders of the liver and bile ducts. We hoped that studies on

the heterogeneity of serum peptidases might help in differentiating between various pathological conditions leading to raised serum peptidase activities. The results obtained have not confirmed these assumptions. Though the presence of certain serum fractions with GGTP and LAP activities was a constant finding in patients with myocardial infarction, and their appearance followed a regular pattern, the diagnostic value of these observations is limited, since a similar pattern of distribution was found in some hepatobiliary diseases (Orłowski, and Szczeklik, 1967). Fortunately, the differentiation between hepatobiliary diseases and myocardial infarction usually offers little difficulty on clinical grounds.

Acute coronary insufficiency should also be considered as a possible cause of raised serum peptidase activities. In our material an increase in GGTP was frequently observed in this group, while the activities of GP and LAP usually remained unchanged. This may suggest that there is a much higher incidence of myocardial necrosis in patients with this clinical presentation than has been previously recognized (Hedworth-Whitty *et al.*, 1967).

The results here presented indicate that the determination of serum peptidase activities, particularly that of GGTP, may be useful as a late enzymatic test of myocardial infarction. This test might be of special interest in cases with an atypical clinical course and equivocal electrocardiographic changes. The determination of the serum peptidases may also be of diagnostic interest in patients admitted to hospital several days after coronary occlusion has occurred, in whom the activities of necrosis-reflecting enzymes have already returned to normal values.

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