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M. D. Pérez-Cárceles · E. Osuna · D. N. Vieira
A. Luna

Usefulness of myosin in the postmortem diagnosis of myocardial damage

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Abstract In some situations the postmortem diagnosis of myocardial infarction is made difficult by the brief course of the fatal episode or by interferences caused by autolysis. In such cases, biochemical indices may provide a useful adjunct to morphological studies. Myosin is the main component of the contractile apparatus of muscle cells, so its determination may well be useful to evaluate myocardial injury. The purpose of the present study was to establish the diagnostic efficacy of postmortem myosin heavy chain determinations using monoclonal antibodies and to compare this data with structural findings used to diagnose acute myocardial ischaemia. We studied 105 cadavers with a mean age of 61.63 ± 2.21 years. Cases were allocated to 1 of 7 diagnostic groups depending on the probable intensity of myocardial damage and cause of death. The highest serum and pericardial fluid values of myosin heavy chains were seen in subjects who showed morphological evidence of myocardial ischaemia. Mean pericardial fluid/serum ratios differed significantly between subjects with and without observable signs of heart damage.

Key words Myocardial ischaemia · Biochemistry · Myosin · Postmortem · Pericardial fluid

Introduction

In some situations the postmortem diagnosis of myocardial infarction is made difficult by the brief course of the fatal episode, which leaves behind no observable structural parameters. Also, the structural markers may be masked by interference due to postmortem autolysis. In such cases, biochemical indices may provide a useful adjunct to morphological studies.

The accuracy of diagnosis of any tests in the postmortem diagnosis of myocardial infarction is largely dependent on the patient population to which it is applied. Increases in the levels of biochemical markers of cardiac injury have better specificity when pathological alterations characteristic of ischaemia are present. It has been suggested (Adams et al. 1993) that an ideal biochemical marker of myocardial injury would a) be found in high concentration in the myocardium, b) not be found in other tissues, even in trace amounts or under pathological conditions, c) be released rapidly and completely after myocardial injury, d) be released in direct proportion to the extent of the injury, and e) persist in plasma for several hours to provide a convenient diagnostic time window but not so long that recurrent injury would not be identified.

Myosin is the main component of the contractile apparatus of muscle cells. Its central role in muscle contraction was first investigated in the 1930s, when myosin was separated from actin and isolated. The myosin molecule consits of 2 heavy chains each with a molecular weight of 200,000, and 2 pairs of dissimilar proteins each with a molecular weight of 20,000 or 26,000, designated myosin light chain I and II. During the 1980s different contractile proteins (myosin light chains, myosin heavy chains, tropomyosin and troponin fragments) were quantified in serum from patients suffering from acute myocardial infarction, and were used in the biochemical diagnosis of myocardial necrosis in clinical practice (Khaw et al. 1976, 1978; Nagai et al. 1979, 1983; Cummins et al. 1981, 1987; Clark-Nolan et al. 1983; Leger et al. 1985, 1990; Katus et al. 1987). The usefulness of these markers is based on the fact that contractile proteins, in contrast to other cytoplasmic proteins, are not normally present in appreciable amounts in the blood circulation (Leger et al. 1990).

Myosin heavy chains are released later than the light chain fraction. Fragments of myosin heavy chains may be detected from the second day after infarction, and levels may remain elevated for 8–10 days (Leger et al. 1990). The relatively long delay in their appearance in relation to

M. D. Pérez-Cárceles · E. Osuna (✉) · A. Luna
Department of Forensic Medicine, School of Medicine, University of Murcia, E-30100 Espinardo, Murcia, Spain

D. N. Vieira
Institute of Legal Medicine, University of Coimbra, P-3000 Coimbra, Portugal

other biochemical markers is one of the most characteristic features of the release kinetics. Although this makes myosin fragments of little use in diagnosing the acute phase of myocardial infarction, these proteins are nonetheless useful in evaluating the prognosis and post-infarction course (Leger et al. 1985). Moreover, during agonal suffering, the decrease of blood perfusion, decreased oxygen concentration, and increased carbon dioxide concentration, as well as other factors, can lead to the release of biochemical markers. For example, Hoberg et al. (1987) reported that myosin light chains are released into the bloodstream when minimal myocardial damage occurs with no clinically evident infarction. In our study we found evidence of minimal myocardial damage with another marker (creatine kinase MB isoenzyme). The molecular weight and release kinetics of this marker lead to elevated levels earlier than myosin heavy chains. The main reason for measuring levels of myosin heavy chains in the present study was that their release is a confirmatory sign of myocardial cell necrosis. The purpose of the present study was to establish the diagnostic efficacy of postmortem myosin heavy chain determinations using monoclonal antibodies and to compare this data with structural findings used to diagnose acute myocardial ischaemia.

Materials and methods

A total of 105 cadavers was studied, and the mean age of the subjects was 61.63 ± 2.21 years (SD 21.71 years). Cases were allocated to 1 of 7 diagnostic groups based on patient records, scene of death, autopsy, toxicological, and histological findings, depending on the probable intensity of myocardial damage and cause of death. The groups were defined as: (A) 24 cases of death from definite myocardial infarction (13 cardiac deaths witnessed); (B) 8 cases with morphological signs of myocardial or cardiac disease where chest trauma was involved in the cause of death; (C) 27 cases with morphological signs of myocardial or cardiac disease, where death was due to violent causes excluding chest trauma; (D) 12 cases with morphological signs of myocardial or cardiac disease: death due to natural causes; (E) 21 cases without morphological signs of myocardial or cardiac disease, where death was due to extensive multiple injuries; (F) 7 cases with no morphological signs of myocardial or cardiac disease and death was due to violent causes excluding trauma; and (G) 6 cases with no morphological signs of myocardial or cardiac disease and death was due to natural causes.

Survival periods varied from a few minutes or less (41 cases) to more than 24 h (35 cases) and in 29 cases the survival time was between 1 and 12 h. Cardiopulmonary resuscitation was used in 10 subjects.

Pericardial fluid samples were obtained from the opened pericardial sac with a sterilized syringe. Serum from femoral vein blood was obtained by needle and syringe puncture before autopsy. Samples were stored at –40° C. All samples were analyzed in duplicate. Creatine kinase MB isoenzyme (CK-MB) in serum and pericardial fluid was determined by UV spectrophotometry and commercial kits (Boehringer Mannheim). Myosin was measured by RIA using a sandwich technique involving 2 antimyosin heavy chain monoclonal antibodies from Sanofi Diagnostic Pasteur SA only in serum and pericardial fluid, but not in vitreous humor, as the first 50 determinations in this material were negative. When initial determinations showed concentrations of markers higher than the clinical range, the samples were diluted with saline and stabilized with 3% albumin to adjust the concentrations to clinical ranges in serum.

To obtain information on the behaviour of myosin and its distribution in pericardial fluid and serum in different diagnostic groups, we calculated the ratio of myosin concentration in pericardial fluid of myosin concentration in serum for each of the 105 cadavers. Histological studies with hematoxylin-eosin (H&E) and acridine orange staining were performed for samples of heart tissues taken from ischaemic or necrotic zones (when a set of macroscopic features in the routine autopsy are present) and from 8 control samples (the middle of the fat-free part of the anterior and posterior walls of the right ventricle; and the border of upper and lower third of the septal, anterior and posterior walls of the left ventricle) standardized for every individual.

When H&E or acridine orange staining were positive we established the diagnosis of myocardial infarction (group A). When the H&E method showed unspecific signs (congestion, interfibrillar edema, fibrosis, etc) and the orange acridine was classified as unclear, the cases were allocated in the groups of subjects with morphological signs of myocardial or cardiac disease (groups B, C and D).

One-way analysis of variance (ANOVA 1) was used to compare mean values between different groups. Statistically significant correlations between different variables and discriminant analysis were also determined.

Results

Table 1 shows the values (mean and SD) of the biochemical parameters in relation to the diagnostic groups. The results of ANOVA 1 and Student's t-test of the values for the different groups are given in Table 2. Figure 1 shows the mean concentrations of myosin heavy chains in serum and pericardial fluid. The highest serum values were found in subjects with morphological signs of heart alter-

Table 1 Mean and (SD) values for the myosin and CK-MB for the 7 groups

	Myosin (μU/l)						CK-MB (U/l)			
	p		s		ratio		p		s	
	x	(SD)	x	(SD)	x	(SD)	x	(SD)	x	(SD)
A	1970	(2958)	420	(550)	8.18	(14.21)	4792	(5843)	3183	(3393)
B	1536	(1953)	1395	(1431)	1.96	(2.06)	1017	(1045)	1353	(996)
C	498	(547)	673	(466)	1.47	(3.52)	2265	(3614)	1517	(1441)
D	314	(202)	458	(537)	1.83	(2.43)	2496	(3116)	3157	(4701)
E	519	(968)	795	(1008)	0.64	(0.86)	1090	(1345)	2051	(1902)
F	340	(412)	410	(376)	1.82	(2.34)	1629	(3124)	2004	(1968)
G	201	(248)	143	(96)	2.15	(2.57)	3508	(3844)	893	(871)

Abbreviations: s = serum; p = pericardial fluid

Table 2 One-way analysis of variance and Student's t-test of the values for the 7 groups.

Variable	gl	F exp.	p	Groups	t	p	Groups	t	p
Myos s	6	2.693	0.018	A–B	3.298	0.001	B–E	–1.992	0.049
				B–C	–2.477	0.014	B–F	–2.626	0.010
				B–D	–2.833	0.005	B–G	–3.201	0.001
Myos p	6	3.0129	0.0096	A–C	–3.2449	0.0016	A–F	–2.3469	0.0209
				A–D	–2.8960	0.0047	A–G	–2.3968	0.0184
				A–E	–3.0027	0.0034			

Abbreviations: Myos = myosin; s = serum; p = pericardial fluid; gl = degrees of freedom; t = Student's t-test; p = probability

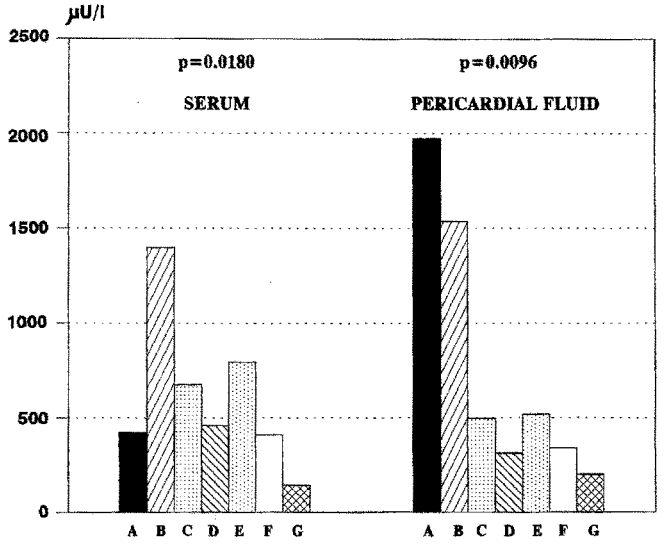

Fig. 1 Mean concentrations of myosin heavy chains in serum and pericardial fluid

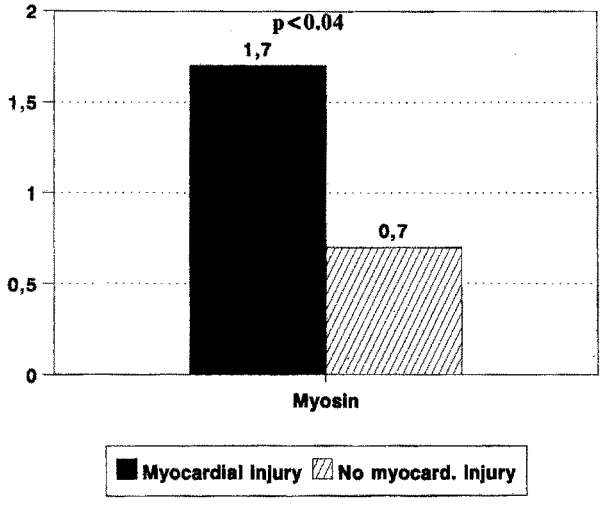

Fig. 2 Mean pericardial fluid/serum ratios of myosin heavy chains in cases with (■) and without (▨) myocardial injury

ations where chest trauma was involved in the cause of death. The differences were greater in pericardial fluid and the highest levels were seen in subjects who had died from definite myocardial infarction. Mean pericardial fluid/serum ratios (Fig. 2). differed significantly ($p < 0.04$)

Table 3 Correlation matrix for the 105 cases studied

	myosin s	myosin p	CKMB s	CKMB p
Diagnostic group	N.S.	0.331	N.S.	0.208
Cardiopulmonary	N.S.	N.S.	N.S.	N.S.
Widespread multiple injuries	0.328	N.S.	0.209	0.234
Chest injuries	0.232	N.S.	N.S.	0.190
Hematoxylin-eosin stained	N.S.	0.248	N.S.	N.S.
Acridine orange stained	N.S.	0.352	0.264	0.347

Abbreviations: s = serum; p = pericardial fluid; CKMB = creatine kinase MB; N.S. = No statistically significant correlation

Table 4 Discriminant analysis choosing the diagnostic category as the grouping variable

Variable Entered	F Value	Approx-imate F Statistic	Degrees of Freedom
Acridine orange	91.4115	91.411	2–102
Hematoxylin and eosin	12.0852	43.444	4–202
Myosin in pericardial fluid	4.1761	31.214	6–200

between subjects with and without observable signs of heart damage.

The correlation matrix (Table 3) shows that serum myosin heavy chain concentrations correlated significantly with widespread multiple injuries ($r = 0.328$) and chest injuries ($r = 0.232$). Myosin heavy chain concentrations in pericardial fluid correlated significantly with the diagnostic group ($r = 0.331$), and with the findings in H&E ($r = 0.248$) and acridine orange-stained tissue sections ($r = 0.352$). For the discriminant analysis we chose the diagnostic category as the grouping variable, establishing 3 groups: A) cases of death from definite myocardial infarction and subjects with morphological signs of myocardial or cardiac disease where chest trauma was involved in the cause of death (32 cases); B) subjects with morphological signs of myocardial or cardiac disease where death was due to violent causes excluding chest trauma or due to natural causes (39 cases); C) subjects without morphological signs of myocardial or cardiac disease (34 cases). Correct classification was found (jackknifed classification) in 74.3% and we found that the findings in H & E

and acridine orange-stained tissue sections and myosin concentrations in pericardial fluid were the variables that best classified the cases (Table 4).

Discussion

The highest serum concentrations of myosin heavy chains were found in cadavers with morphological signs of heart alterations or injuries involving widespread muscle destruction. This finding is in agreement with results reported by Leger et al. (1985, 1990) and Fechner et al. (1991). In pericaridal fluid, myosin heavy chain concentrations were significantly elevated in cases of sudden death due to heart-related causes. The release kinetics of myosin may account for this finding (Leger et al. 1985). Myosin concentrations in pericardial fluid correlated significantly with the degree of cardiac damage and with the findings in H&E and acridine orange-stained tissue sections. We therefore consider myosin heavy chains to be a reliable biochemical marker in the diagnosis of cardiac necrosis. The pericardial fluid/serum ratio of myosin concentrations was, however, clearly the best indicator of widespread muscle damage or cardiac necrosis. The delayed release kinetics of myosin, together with interference from myosin released by skeletal muscle in response to multiple injuries, rule out serum as a useful source of information in postmortem analysis to discriminate myocardial infarction. Because myosin is released more rapidly in pericardial fluid, this material is more suitable than serum for the postmortem diagnosis of cardiac necrosis.

We found no correlation between myosin concentrations in serum or pericardial fluid and the use of cardiopulmonary resuscitation. Also, we found no statistically significant correlation between myosin heavy chains and CK-MB concentrations. The delayed release kinetics of myosin can explain this fact. Although myoglobin and CK-MB are sensitive markers of use in ruling out cardiac injury regardless of its origin (Luna et al. 1982, 1983; Cairns et al. 1983; Grenadier et al. 1983; Stone and Willerson 1983; Stewart et al. 1984; Lee and Goldman 1986; Hoberg et al. 1987; Lee et al. 1987; Isakov et al. 1988), myosin is an excellent marker of severe myocardial injury. Higher concentrations of myosin are often accompanied by observable morphological signs of heart damage, and are thus useful in diagnosing sudden death due to heart-related causes.

However, like any other biochemical marker, myosin alone is of limited value, and should be considered together with a wider body of reference data that includes morphological and other biochemical findings. Because we found no published studies of biochemical and morphological findings in cadavers against which to compare our present observations, it is difficult to discuss and interpret some of our findings. However, from a practical standpoint, our data suggest several conclusions and recommendations for the use of myosin heavy chain concentrations in the postmortem diagnosis of myocardial necrosis.

Because of the structural characteristics of the myosin molecule and its location in the cell, the release of this molecule requires that cellular injury be severe. This is in contrast to other molecules such as myoglobin and CK-MB, that can be released during the intense agonal process that precedes death and cardiac injury occurs with a number of local and general alterations.

In the present study myosin, a relatively stable molecule, was detected in appreciable amounts throughout postmortem intervals of different durations. In addition to its structural features, the cellular location and resistance to postmortem autolysis is particularly useful in the postmortem diagnosis of myocardial infarction.

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