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Myocardial ischaemia and the inflammatory response: release of heat shock protein 70 after myocardial infarction

B Dybdahl, S A Slårdahl, A Waage, P Kierulf, T Espevik, A Sundan

Objectives: To test the hypothesis that heat shock protein (Hsp) 70 may be released into the circulation after acute myocardial infarction (AMI) by exploring the kinetics of Hsp70 release and the relations between Hsp70 and markers of inflammation and myocardial damage in AMI.

Design: Blood samples from 24 patients were prospectively collected through to the first day after AMI. Hsp70, interleukin (IL) 6, IL-8, and IL-10 in serum were measured by enzyme linked immunosorbent assay (ELISA).

Results: Median Hsp70 concentrations in AMI patients measured at arrival, six hours thereafter, and the following morning were 886, 868, and 607 pg/ml, respectively. These concentrations were all significantly different from those of the control patients with angina with a median serum Hsp70 concentration of 306 pg/ml. Peak Hsp70 correlated with creatine kinase (CK) MB \( (r = 0.62, p < 0.01) \) and cardiac troponin T \( (r = 0.58, p < 0.01) \). Furthermore, serum Hsp70 correlated with IL-6 and IL-8 at six hours \( (r = 0.60, p < 0.01 \text{ and } r = 0.59, p < 0.01, \text{ respectively}) \).

Conclusions: In this study, Hsp70 was rapidly released into the circulation after AMI. Circulating Hsp70 is suggested as a marker of myocardial damage. In addition, Hsp70 may have a role in the inflammatory response after AMI.

Methods

The present study conforms to the principles outlined in the Declaration of Helsinki.

Patients and controls

From September 2001 through August 2002, 28 patients were enrolled in this prospective study at Trondheim University (St Olav) Hospital, Trondheim, Norway. The inclusion criteria were chest pain for more than 20 minutes (but less than 12 hours) and strong clinical suspicion of AMI with or without ECG changes. The diagnosis of AMI was secured by echocardiography and the typical rise and fall of the biochemical markers cardiac troponin T (cTnT) and CK-MB, in addition to the presence of ischaemic symptoms. The patients were enrolled at arrival in the emergency unit, and

Abbreviations: AMI, acute myocardial infarction; CK, creatine kinase; CRP, C reactive protein; cTnT, cardiac troponin T; ELISA, enzyme linked immunosorbent assay; Hsp, heat shock protein; IL, interleukin; PCI, percutaneous coronary intervention; TLR, toll-like receptor
Blood sampling

Blood was sampled through venepuncture immediately after admission to the emergency unit, after six hours, on the following morning (12–15 hours after test 2), and thereafter as needed. Routine tests measuring cTnT, CK-MB, CRP, and haematological parameters were done by the department of clinical chemistry, Trondheim University Hospital. Through the first day, an extra blood sample was taken concomitantly with the routine tests. This sample was centrifuged after 30 minutes of coagulation and serum was aliquoted and frozen in several vials at −80°C until analysed for Hsp70, IL-6, IL-8, and IL-10.

Serum analyses

Serum Hsp70 was measured with a StressXpress Hsp70 enzyme linked immunosorbsent assay (ELISA) kit (Stressgen Biotechnologies Corp, Victoria, British Columbia, Canada) with a sensitivity of 200 pg/ml and interassay and intra-assay coefficients of variation < 10%. IL-6 and IL-8 were measured with Quantikine Human Immunoassays (R&D Systems Inc, Minneapolis, Minnesota, USA) with sensitivities of 0.70 pg/ml and 10 pg/ml, respectively. Serum IL-10 was analysed with Biosource IL-10 EASIA Kit (Biosource Europe SA, Nivelles, Belgium) with a sensitivity of 1 pg/ml. cTnT and CK-MB were measured by means of electrochemiluminescence immunoassay technology on an Elecsys 2010 Analyser.

Table 1

Baseline patient data and clinical characteristics (n = 24)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.0 (49.5, 63.0)</td>
</tr>
<tr>
<td>Male/female</td>
<td>19/5</td>
</tr>
<tr>
<td>Anterior MI</td>
<td>9</td>
</tr>
<tr>
<td>Inferior MI</td>
<td>13</td>
</tr>
<tr>
<td>Non-Q wave MI</td>
<td>2</td>
</tr>
<tr>
<td>Time from pain onset to hospital admission (hours)</td>
<td>3.0 (1.0, 4.4)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>50 (50, 65)</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>11</td>
</tr>
<tr>
<td>Primary PCI</td>
<td>12</td>
</tr>
<tr>
<td>Rescue PCI</td>
<td>1</td>
</tr>
<tr>
<td>In-CCU stay (days)</td>
<td>2 (1.0, 2.0)</td>
</tr>
<tr>
<td>In-hospital stay (days)</td>
<td>7 (6.0, 8.0)</td>
</tr>
<tr>
<td>30 day mortality</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are median (first and third quartiles) or number of patients.

CCU, coronary care unit; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention.

Figure 1

Serum cardiac troponin T (cTnT, n = 24) after acute myocardial infarction (AMI), individual and median concentrations (horizontal bars). The median concentrations at the three time points were 0.02, 4.32, and 4.33 µg/ml. cTnT concentration increased significantly from arrival to six hours later and from arrival to the following day. Log10 scale is used on the vertical axis. One blood test from the third sampling was lost.

Figure 2

Serum creatine kinase MB (CK-MB) in patients with AMI (n = 24). Individual concentrations and medians are shown. Median serum concentrations at the three time points were 4.7, 178.3, and 97.3 µg/ml. The increases in CK-MB from hospital admission to both six hours later and the following day were significant. Log10 scale is used on the vertical axis. One sample from day 1 was lost.
with detection ranges of 0.01–25.00 mg/l and 0.10–500 mg/l, respectively. Cut off values in the diagnosis of AMI were determined to be 0.10 mg/l for cTnT and 0.010 mg/l for CK-MB. Routine hospital analyses were used for quantification of CRP (detection limit 5 mg/l), leucocyte counts, high density lipoprotein cholesterol, total cholesterol, and triglycerides.

Statistical analyses
Owing to the non-Gaussian distribution of most data, we used non-parametric tests and median values with quartiles. We used the Mann-Whitney test to compare cases with controls and to compare subgroups. We used the Wilcoxon signed ranks test to compare two related samples. Comparisons within groups over time were made by means of the Friedman test and Dunn’s multiple comparison test. Differences were considered significant p < 0.05. For correlation analyses, we used Spearman’s rho.

RESULTS
Hsp70 and myocardial damage
Serum Hsp70 in patients with AMI were significantly higher than in the control group at all time points (fig 3). Hsp70 concentrations in patients with AMI peaked six hours after their arrival (median 868 pg/ml). In comparison, the control group had a median serum Hsp70 concentration of 306 pg/ml. Hsp70 decreased significantly in the patients with AMI from the day of arrival to the following morning, in contrast to cTnT (fig 1) and CK-MB (fig 2). Median serum cTnT was at maximum at 8 am the following day (4.33 µg/l) in patients with AMI. Moreover, their peak median CK-MB concentration was 178.3 µg/l six hours after arrival at the hospital. Control patients had a median cTnT < 0.01 µg/l and median CK-MB 1.4 µg/l, both significantly lower (p < 0.01) than the corresponding concentrations in patients with AMI. Median time from pain onset to hospital admission in our patients was 4.8 hours in patients undergoing primary PCI versus 4.0 hours in patients not treated with primary PCI (p < 0.05).

The peak Hsp70 concentration correlated with time from pain onset to hospital admission (r = 0.54, p < 0.01). Furthermore, peak Hsp70 in serum correlated with the peak concentrations of the markers of myocardial necrosis CK-MB (r = 0.62, p < 0.01) and cTnT (r = 0.58, p < 0.01) (fig 4A, B) measured at the same time points. Hsp70 both at arrival and at six hours correlated with cTnT measured on day 1 (r = 0.51, p < 0.05 and r = 0.67, p < 0.01, respectively), illustrating the more rapid release of Hsp70. Interestingly, left ventricular ejection fraction correlated negatively with peak Hsp70 concentration (r = –0.52, p < 0.05) (fig 4C).

Studying each individual patient carefully, we discovered that there were none that had low serum cTnT combined with high Hsp70 concentrations. Patients with AMI and high Hsp70 concentrations at arrival usually retained a higher serum Hsp70 concentration during the first day compared.
with patients with lower initial concentrations. One of our patients, a 63 year old woman who had a large myocardial infarction, had Hsp70 concentrations of 2351, 2120, and 1001 pg/ml, respectively, measured at admission, six hours later, and the morning after. A second example is a 51 year old man with an occluded right coronary artery, experiencing his first myocardial infarction, with corresponding serum Hsp70 concentrations of 811, 1090, and 540 pg/ml, respectively.

**Systemic inflammation**

Control patients had CRP < 5 mg/l, median IL-6 2.4 pg/ml, and median IL-8 13.2 pg/ml. Table 2 shows supplementary laboratory values for the patients with AMI. There was a significant increase in CRP from admission to the following morning (p < 0.01). Figures 5 and 6 show the results of serum IL-6 and IL-8 measurements, respectively. The IL-6 median concentration peaked at 14.1 pg/ml the day after arrival. Serum IL-6 concentrations in controls were significantly (p < 0.01) lower than in patients with AMI at all time points. For patients with AMI, there was a significant increase in IL-6 from the day of arrival to the day after, thereby having kinetics different from Hsp70. In contrast, the IL-8 concentration was quite low and did not increase during the first day after AMI. Median serum IL-8 was 15.3 pg/ml measured the morning after the insult.

We detected a strong correlation between the proinflammatory IL-6 and Hsp70 measured at six hours after arrival at the hospital (r = 0.60, p < 0.01). There was still a correlation between IL-6 and Hsp70 the following day, though it was weaker (r = 0.44, p < 0.05). At six hours, IL-6 also correlated with CK-MB (r = 0.58, p < 0.01) and cTnT (r = 0.48, p < 0.05), indicating a possible relation between myocardial damage and systemic inflammation. The chemokine IL-8 correlated with Hsp70 at six hours after hospital admission (r = 0.59, p < 0.01) but not the day after. IL-8 correlated with CK-MB six hours after hospital admission (r = 0.45, p < 0.05) but no correlation with cTnT was detected. In addition, CRP measured on day 1 after the AMI correlated with the peak Hsp70 (r = 0.56, p < 0.01).

Four patients had detectable concentrations of the anti-inflammatory IL-10 at arrival at the hospital (range 6.6–16.1 pg/ml). IL-10 was not further statistically analysed because of the small number of patients with detectable concentrations of this cytokine.

**DISCUSSION**

The main findings in the present study were increased serum Hsp70 concentrations in patients with AMI as compared with patients with angina pectoris. Furthermore, peak serum Hsp70 concentrations correlated both with markers of myocardial necrosis and with proinflammatory markers. Lastly, in patients with AMI serum Hsp70 concentration increased and declined more rapidly than the well known markers of myocardial necrosis, cTnT and CK-MB. We are not aware of previously published studies on circulating Hsp70 after AMI.

**Hsp70 and myocardial necrosis**

All of our patients had myocardial necrosis as shown by the increase in cTnT and CK-MB, as well as by typical echocardiographic findings. cTnT and CK-MB are well known markers of myocardial necrosis. As a group, our patients...
with AMI had moderate peak cTnT concentrations, although with substantial individual differences. We clearly detected a correlation between markers of myocardial damage and serum Hsp70 in our patients. This may indicate that the Hsp70 concentration is related to the size of the infarction and therefore to the prognosis, even though the relation between the size of the infarction and markers of necrosis is less obvious after a revascularisation procedure. On the other hand, the observation that patients with higher serum Hsp70 concentrations had lower left ventricular ejection fractions the first day after AMI supports the idea that the Hsp70 concentration is related to the size of the infarction. Thus, we suggest that circulating Hsp70 after AMI may be a marker of myocardial damage.

**Hsp70 kinetics**

An important difference between the established markers of myocardial necrosis, cTnT and CK-MB, and Hsp70 is their kinetics: Hsp70 appears to be more rapidly released and cleared from the circulation. The biological half life of Hsp70 has been reported to be about 18 hours. In AMI, cTnT is still high several days after the primary insult. We measured Hsp70 may be of value in detecting reinfarction during the first few days after the primary AMI.

**Systemic inflammatory response in AMI**

An inflammatory response appeared after AMI in the study patients. We detected an increase in IL-6 and CRP during the first day, in accordance with previous studies. We observed that IL-8, important in chemotaxis and angiogenesis after reperfusion in AMI, did not change significantly during the first day after AMI. One patient (who died on day 2), however, had high IL-8 concentrations. Other studies have had conflicting results on the role of IL-8 in acute coronary syndromes.

Furthermore, as shown in previous studies, increased serum concentrations of the anti-inflammatory IL-10 are associated with a more favourable prognosis for patients with acute coronary syndromes and increased CRP, and patients with unstable angina pectoris may have lower concentrations of IL-10 than patients with chronic stable angina. We detected IL-10 in only four of our 24 patients. Thus, for statistical reasons, we should not draw conclusions about the relation between Hsp70 and IL-10 from this investigation.

**Hsp70 and the immune system**

The median serum Hsp70 concentration after AMI in our patients was not very high. Whether this amount of systemic Hsp70 is enough to stimulate circulating cells of the immune system remains to be elucidated. In vitro, concentrations of Hsp70 up to 0.1–10 μg/ml have been used to stimulate monocytes. Nevertheless, our patients’ results may support a relation between Hsp70 and a systemic inflammatory response. Besides the possibility of a systemic Hsp70 effect, Hsp70 may have an important role locally in the heart, not only as a chaperone but also as a signalling molecule.

Expression of TLR-4 is reported in the human myocardium. As a putative TLR ligand, Hsp70 has been suggested to activate innate immunity in injured myocardium, coronary arteries, and monocytes in vivo. However, the mechanism by which Hsp70 may activate innate immunity is unknown. Results from a recent study show that recombinant preparations of human Hsp70 may be contaminated by endotoxin, a known TLR-4 ligand. An alternative interpretation of existing data may be that Hsp70 has an endotoxin binding function, delivering endotoxin to receptor complexes including TLR-4 on antigen presenting cells.

Importantly, Hsp70 may activate adaptive immunity as well through the CD91 receptor on antigen presenting cells, thereby inducing Th2-type CD4 T cells, synthesising IL-10 among others. As such, Hsp70 may be part of an immunomodulatory response that has the potential to control proinflammatory responses.

**Study limitations**

It remains to be clarified whether Hsp70 is a valid clinical marker for cardiac damage. The sample size in the study is too small to allow any definite conclusions in this regard. Furthermore, our patients had small myocardial infarctions. This appears to be a consequence of early and intense treatment. With larger infarctions, we would expect to detect even larger differences in serum Hsp70 than we did between patients with angina and patients with AMI. Nevertheless, the main findings of this pilot study may generate hypotheses and support further investigation.

**Conclusions**

To summarise, Hsp70 was rapidly released and cleared from the circulation after AMI and may be suggested as an additional marker of myocardial damage. Besides its known function in intracellular repair processes, Hsp70 may activate the immune system, thus having a role in both local and systemic inflammatory responses after AMI.

**ACKNOWLEDGEMENTS**

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There is no conflict of interest.

**REFERENCES**


An 80 year old man with a history of abdominal aortic aneurysm, repaired 10 years previously, presented to the emergency room with a four hour history of severe epigastric pain. Examination demonstrated a distended, tender, but non-rigid abdomen. ECG was unremarkable. Shortly after arrival the patient suffered a sudden onset of massive haematemesis requiring replacement of large volumes of intravenous colloid and blood. Following successful resuscitation urgent endoscopy was undertaken, but failed to demonstrate a bleeding point because of large amounts of fresh blood in the stomach. Abdominal multidetector computed tomography (CT) (Siemens Forchheim, Somatom plus 4) was then performed. Axial images of the abdomen (panel A), at a level just inferior to the renal arteries, showed intravenous contrast medium in a slightly aneurysmal abdominal aorta (black solid arrow). Intravenous contrast medium is also seen outlining the inner wall of the third part of the duodenum (white arrow). An aortoenteric fistula is clearly identified between the two structures (black dashed arrow). Early renal cortical contrast enhancement is seen in the left kidney demonstrating preservation of blood supply. On a sagittal maximal intensity projection reconstruction (panel B), the aortoenteric fistula is once again demonstrated (black arrow) connecting the abdominal aorta to the third part of duodenum. The latter is outlined by intravenous contrast medium (white arrow).

Immediately following CT the patient was sent to the operating room, but rapidly deteriorated during transit and further attempts to resuscitate were unsuccessful.

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