Is Hsp27 a marker of myocardial ischaemia?

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Abstract

Background: Heat shock protein (Hsp) 27 expression in cardiomyocytes increases in response to ischaemia. The extracellular release of Hsp27 from cardiomyocytes is proportional to its intracellular levels.

Aim: To assess the influence of significant coronary artery disease (CAD), which by definition results in chronic myocardial ischaemia, on blood serum levels of Hsp27.

Methods: Blood serum levels of Hsp27 in 62 patients with at least 50% lumen diameter narrowing in at least one main epicardial coronary artery on angiography and in 21 controls with normal coronaries were measured.

Results: Patients with CAD tended to have higher serum level of Hsp27 than controls [0.463 (0.158-0.809) vs. 0.184 (0.099-0.337) ng/ml, p = 0.084]. Serum Hsp27 level in patients with CAD affecting more than a single vessel was significantly increased [0.529 (0.192-1.004) ng/ml] compared with controls (p = 0.035) and with one artery narrowed [0.276 (0.087-0.549) ng/ml, p = 0.041]. No correlation between Hsp27 serum levels and severity of coronary narrowings assessed by Gensini score was found (r = 0.21, p = 0.11).

Conclusions: Serum level of Hsp27 seems to be a potential marker of myocardial ischaemia caused by advanced 2- or 3-vessel CAD.

Key words: coronary artery disease, coronary atherosclerosis, coronary flow reserve

Introduction

Heat shock protein Hsp27 (27 kDa) belongs to a family of heat shock proteins with a low molecular mass and is typical for cardiovascular cells such as cardiomyocytes or endothelial cells [1]. Heat shock protein 27 takes part in cell protection against various stress factors, including ischaemic stress [2-4]. Heat shock protein 27 prevents cytoskeleton fragmentation and contributes to intracellular increase of glutathione concentration, diminishing sensibility of cardiomyocytes to oxidative stress caused by ischaemia and reperfusion [4, 5]. Cardioprotective effects of Hsp27 depend mainly on ischaemia-induced extensive phosphorylation of this protein with moderate increase of total Hsp27 concentration in the cell [3, 4].

Like other heat shock proteins, Hsp27 is secreted outside the cell and can be found in a soluble form in the blood (sHsp27). It is assumed that the level of extracellular secretion of Hsp27 and therefore its extracellular concentration (e.g. in the blood) are proportional to its intracellular concentration [6]. Therefore in patients with permanent or recurrent myocardial ischaemia, as in the case of significant coronary artery disease (CAD) defined as the presence of at least 50% lumen reduction in at least one epicardial coronary artery [7], Hsp27 concentrations in cardiomyocytes should be higher than in patients with normal myocardial perfusion. In effect, myocardial release of Hsp27 into blood in subjects with CAD should be increased in comparison to those without significant myocardial ischaemia and the value of Hsp27 concentration in the blood could be a marker of CAD.

Until now (the beginning of 2009) there has been only one report available on Hsp27 concentration in the blood in patients with CAD. Heat shock protein 27 concentration in the blood was analysed in patients with acute coronary syndromes and with chronic stable CAD. Concentration of Hsp27 was higher in patients with ACS in comparison to controls, while patients with stable CAD had insignificantly higher concentration of Hsp27. A limitation of that study was a lack of angiographic diagnostics in the group of stable CAD as the diagnosis of CAD was made solely on the basis of typical medical history [8].

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The current study for the first time assesses the Hsp27 blood concentration in patients with angiographically confirmed stable CAD and correlates Hsp27 concentration with the severity of CAD.

Methods

Study population

The study group consisted of 62 patients with angiographically confirmed CAD, 50 men and 12 women, 54.0 ± 5.8 years of age. The control group included 21 patients, 16 men and 5 women, 54.1 ± 5.9 years of age with no changes in the coronary angiogram (Table I). All patients from the study and the control group were referred for coronary angiography because of recurrent chest pain. Study recruitment was performed between March 2006 and December 2006. A study inclusion criterion in the CAD group was a stable clinical course of CAD during the 2 months preceding the study and at least 50% narrowing in at least one major epicardial coronary artery. The control group included patients without any changes in coronary angiography (coronary arteries without narrowing and smooth, i.e. without marginal unevenness). An additional inclusion criterion in the control group was an exclusion of microvascular coronary dysfunction based on a negative electrocardiographic exercise test (ST-segment depression of less than 1 mm in any of the 12 ECG leads) or on a satisfactory coronary reserve (described below). As a result the control group consisted of patients with excluded ischaemic etiology of chest pain.

Other exclusion criteria were similar in the study and in the control group and included situations potentially influencing Hsp27 concentration in the blood. These were: 1. uncontrolled diabetes (fasting plasma glucose or accidental plasma glucose during hospitalisation exceeding 140 mg% or 200 mg% or HbA1c > 7.5% respectively); 2. uncontrolled hypertension (> 160/90 mmHg in more than 50% of measurements during the preceding month); 3. chronic heart failure > NYHA class I and/or left ventricular ejection fraction (LVEF) < 40% in the current echo-cardiography; 4. haemodynamically significant valvular disease; 5. acute infection during the preceding 3 weeks; 6. history of chronic infectious disease (e.g. COPD, rheumatological diseases); 7. anaemia (Hb below 11 g%); 8. neoplasm; 9. history of ischaemic stroke or other ischaemic states besides CAD. The study was approved by

Table I. Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coronary artery disease</th>
<th>Normal coronary arteries</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women (n)</td>
<td>50/12</td>
<td>16/5</td>
<td>NS</td>
</tr>
<tr>
<td>Age [years]</td>
<td>54.0 (43-66)</td>
<td>54.1 (43-69)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>28.0 (20.0-30.0)</td>
<td>26.7 (23.0-30.0)</td>
<td>NS</td>
</tr>
<tr>
<td>SBP [mmHg]</td>
<td>125.1 (110-140)</td>
<td>129.5 (110-140)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>DBP [mmHg]</td>
<td>79.0 (60-90)</td>
<td>81.9 (70-90)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol [mmol]</td>
<td>4.76 (2.35-8.46)</td>
<td>4.95 (3.49-7.65)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol [mmol]</td>
<td>2.68 (1.03-6.41)</td>
<td>2.90 (1.71-5.38)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol [mmol]</td>
<td>1.32 (0.79-3.59)</td>
<td>1.36 (0.87-0.98)</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin [mmol]</td>
<td>9.0 (6.95-10.6)</td>
<td>9.0 (7.51-10.12)</td>
<td>NS</td>
</tr>
<tr>
<td>WBC [10³/μl]</td>
<td>7.3 (4.9-11.4)</td>
<td>6.7 (4.4-10.7)</td>
<td>NS</td>
</tr>
<tr>
<td>hsCRP [mg/l]</td>
<td>2.62 (0.34-4.88)</td>
<td>3.39 (0.14-12.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine [μmol]</td>
<td>84.0 (39.8-119.3)</td>
<td>75.1 (51.3-97.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting glucose [mmol]</td>
<td>5.2 (4.2-7.8)</td>
<td>5.3 (4.2-5.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular EF (%)</td>
<td>54.1 (40.0-64.0)</td>
<td>59.2 (50.0-65.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>DM (n)</td>
<td>5</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>HA (n)</td>
<td>29</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>16</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>CFR</td>
<td>-</td>
<td>2.34 (2.04-2.97)*</td>
<td>-</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>25 (40)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Previous PTCA, n (%)</td>
<td>19 (30)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: BMI – body mass index, LDL – low density lipoprotein, HDL – high density lipoprotein, WBC – white blood cell count, hsCRP – high-sensitivity C-reactive protein, SBP – systolic blood pressure, DBP – diastolic blood pressure, DM – diabetes mellitus, HA – arterial hypertension, EF – ejection fraction, CFR – coronary flow reserve, NS – non-significant (p ≥ 0.05). Data are presented as medians and interquartile ranges, * n = 8
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the Bioethical Committee. All patients gave written informed consent for participation in the study.

**Coronary angiography**

Coronary angiography was performed using a standard technique. Angiograms were analysed by two experienced interventional cardiologists. Objective quantitative analysis using dedicated software was applied in equivocal cases. The extent of CAD was assessed according to the number of major epicardial arteries with at least 50% lumen diameter reduction [9] and in accordance with this classified as 1-, 2- or 3-vessel disease. Severity of CAD was estimated using the Gensini scale [10].

**Assessment of Hsp27 concentration in blood**

Blood for the Hsp27 assessment was drawn from the peripheral vein before coronary angiography or at least 24 h after coronary angiography (sampling after coronary angiography was limited to patients without one-time coronary angioplasty). Blood was drawn always between 8:00 and 10:00 a.m. at fasting to Vacuette test-tubes including activator of thrombosis. Blood was centrifuged at 3000 rotations/min for 10 min within 2 h of sampling at room temperature to separate plasma and morphotic elements. Plasma was collected and frozen in two approximately 100 μl portions at –70°C for 2-3 months until Hsp27 assessment. Heat shock protein 27 concentration assessment was performed using a commercially available ELISA kit (QIA119, Calbiochem, USA) following the provided manual. The range of Hsp27 concentrations measured by the test was 0.02 ng/ml to 1 ng/ml. Inter- and intra-test variability of Hsp27 assessment was lower than 10%.

**Assessment of coronary flow reserve**

Coronary flow reserve (CFR) was measured to exclude microvascular coronary disease in patients with normal coronary arteries on angiography and positive electrocardiographic exercise test. Measurement of CFR was performed by assessing flow in the left anterior descending (LAD) coronary artery at rest and during intravenous infusion of adenosine. Blood flow in the distal segment of the LAD was demonstrated by transthoracic echocardiography using a Vivid 7 machine (GE Medical Systems, USA) working in the Doppler mode, allowing optimal coronary flow visualisation. The studies were performed by an experienced echocardiographer. After localisation of the distal LAD segment flow was visualised using colour Doppler in the 3-chamber apical projection modified by the delicate counter clockwise rotation of the ultrasonic head and its slight medial angulation. This method allowed fast and accurate localisation of the distal LAD segment. Subsequently, a pulsatile Doppler gate was placed in the distal LAD segment to register blood flow velocity at rest and during myocardial hyperaemia caused by a 2-minute intravenous infusion of adenosine at 140 μg/kg/min (Adenoscor, Sanofi-Synthelabo, France). All studies were registered digitally and CFR was calculated off-line as the ratio of maximal diastolic velocity during hyperaemia and at rest. Coronary flow reserve cut-off value ≥ 2.0 was used to exclude a microvascular coronary disease [11, 12] (Table I).

**Statistical analysis**

All data are presented as medians and interquartile ranges. Statistical analysis was performed using STATISTICA version 5. The Mann-Whitney test or Kruskal-Wallis test for multiple comparisons were used to compare quantitative variables. Qualitative variables were analysed with the χ² test. Correlations were studied with the Spearman test. Statistical significance was set at p < 0.05.

**Results**

The study and the control group differed in terms of systolic blood pressure, LVEF and the number of previous coronary incidents (Table I). Prior to the study, patients with normal coronary arteries were less frequently treated with beta-blockers and more frequently treated with long-acting nitrates in comparison to patients with CAD (Table II). In the CAD group 16 patients had 1-vessel disease, 28 had 2-vessel disease and 18 had significant narrowing of 3 arteries. Median Hsp27 concentration in plasma and its interquartile range in the CAD group were 0.463 (0.158-0.809) ng/ml, with a trend toward higher values in comparison to the control group [0.184 (0.099-0.337) ng/ml, p = 0.084]. Multiple comparisons of Hsp27 concentration in subjects from the control group and patients with 1-, 2-, 3-vessel CAD did not demonstrate...

<table>
<thead>
<tr>
<th>Medication</th>
<th>Coronary artery disease [%]</th>
<th>Control group [%]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-blocker</td>
<td>100</td>
<td>90</td>
<td>0.01</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitor</td>
<td>88</td>
<td>80</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin receptor blocker</td>
<td>10</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Statin</td>
<td>100</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrates</td>
<td>46</td>
<td>90</td>
<td>0.001</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>100</td>
<td>100</td>
<td>NS</td>
</tr>
</tbody>
</table>
significant differences ($p = 0.056$) (Figure 1). However, after exclusion of patients with 1-vessel CAD from the analysis, Hsp27 concentration in the CAD group [0.529 (0.192-1.004) ng/ml, $n = 46$] was significantly higher than in the control group ($p = 0.035$). Likewise, Hsp27 concentration analysis within the CAD group with respect to the number of narrowed coronary arteries showed lower protein concentrations in patients with 1-vessel CAD [0.276 (0.087-0.549) ng/ml, $n = 16$] in comparison to patients with 2- and 3-vessel CAD together ($p = 0.041$).

The severity of CAD in the whole CAD group estimated using the Gensini scale was 30.7 (13.0-44.0). There was no correlation between the grade of CAD severity and Hsp27 concentration in plasma ($r = 0.21$, $p = 0.11$) (Figure 2).

**Discussion**

Coronary artery disease is defined as the presence of 20-30% lumen diameter reduction in at least 1 coronary artery in coronary angiography [9]. However, only 50% or more reduction of lumen diameter is regarded as a cause of myocardial ischaemia and therefore at least 50% stenosis is used to diagnose significant CAD [7]. The current study for the first time assessed Hsp27 concentration in plasma in patients with angiographically confirmed significant CAD and in patients with normal coronary angiogram and at the same time with normal coronary microcirculation. Therefore, the presented study allows the Hsp27 concentration to be assessed not only in patients with confirmed atherosclerosis of the coronary arteries, but primarily in those with myocardial ischaemia, which was the main aim of the study.

Heat shock protein 27 plays a key role in the ischaemic conditioning of the myocardium and limits the progress of oxidative stress caused by reperfusion [2, 13-15]. The protein stabilises the cellular cytoskeleton under stress conditions [16, 17] and counteracts proapoptotic factors [18]. It has been shown that a large number of various stress factors, including ischaemic stress, lead to increased expression of Hsp27 in the cell [3, 4, 19]. This has been confirmed by the observation of increased Hsp27 concentration in cardiomyocytes of patients with ischaemic cardiomyopathy [20].

Our study suggests a trend toward higher Hsp27 concentration in plasma in patients with significant CAD in comparison to those without changes in coronary arteries. Furthermore, exclusion of patients with 1-vessel disease from the analysis (which is usually a condition causing the least extensive myocardial ischaemia) demonstrated that Hsp27 concentration in plasma in patients with CAD is significantly higher than in control subjects. Concentration of Hsp27 was also higher in the group of patients with 2- and 3-vessel CAD in comparison to patients with 1-vessel disease. As the number of coronary arteries with significant stenosis influences the extent of myocardial ischaemia, the results presented above demonstrate a close relationship between Hsp27 concentration in plasma and the mass of ischaemic myocardium. The aforementioned relation should not be surprising, as the intracellular concentration of total Hsp27 increases only moderately under ischaemic conditions. In effect, a relatively small myocardial mass supplied by a single coronary artery may not be sufficient to influence the extracellular concentration of Hsp27 (Hsp27 concentration in plasma). On the other hand, the lack of correlation between Hsp27 concentration in plasma and the severity of CAD assessed with the Gensini scale, which is an angiographic surrogate of myocardial ischaemia extent, suggests a weak or even no relation between Hsp27 concentration and the extent of myocardial ischaemia. However, the fact of the presence of collateral circulation should be considered at least in some patients. In this situation the Gensini scale does not reliably predict...
the extent of myocardial ischaemia, which is generally overestimated. Therefore, the lack of correlation between the Gensini scale and Hsp27 concentration does not exclude the lack of a relationship between the protein and the extent of myocardial ischaemia.

Moreover, the baseline concentration of Hsp27 in plasma is characterised by a marked interpersonal variability [21]. Therefore, a relative increase of Hsp27 concentration instead of a single assessment would allow one to better estimate the extent of myocardial ischaemia. Another issue related to the assessment of Hsp27 concentration in patients with CAD, which cannot be omitted, is the fact that Hsp27 is produced not only in cardiomyocytes but also in the endothelial cells and possibly in the smooth muscle cells. Previous studies have demonstrated that atherosclerotic plaques include less Hsp27 than a healthy vascular wall. Furthermore, it has been suggested that patients with asymptomatic (probably non-stenotic) atherosclerosis have a lower Hsp27 concentration in plasma in comparison to those without atherosclerotic changes in the arteries [8, 22]. Therefore, a small vascular leak of Hsp27 into the blood may underestimate an increase of Hsp27 concentration in the blood caused by excretion of the protein from the ischaemic myocardium.

Conclusions

The presented results suggest that Hsp27 concentration in the blood is a marker of severe myocardial ischaemia typical for 2- or 3-vessel CAD. Further studies on large groups of patients and possibly using techniques such as cardiac scintigraphy or positron emission tomography, allowing a quantitative assessment of myocardial ischaemia in correlation with Hsp27 concentration in the blood, are needed to confirm this suggestion.

References

Białko szoku cieplnego Hsp27 markerem niedokrwienia miokardium?

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Streszczenie

Wstęp: Ekspresja białka szoku cieplnego Hsp27 wzrasta w kardiomiocytach poddanych niedokrwieniu. Wiadomo, że wzrost ekspresji wewnątrzkomórkowej Hsp27 powoduje jego zwiększone uwalnianie z komórki do płynów ustrojowych.

Cel: Ocena wpływu istotnej choroby wieńcowej (ChW), która z definicji wiąże się z przewlekłym niedokrwieniem miokardium, na stężenie Hsp27 w krwi obwodowej.

Metody: Przy użyciu komercyjnie dostępnego testu ELISA (Calbiochem, Stany Zjednoczone) zmierzono stężenie Hsp27 w surowicy krwi obwodowej 62 pacjentów z potwierdzoną koronarograficznie przynajmniej 50-procentową stenozą w przynajmniej jednej tętnicy nasierdzowej i w surowicy 21 osób z prawidłowymi tętnicami wieńcowymi w badaniu angiograficznym. Kryteriami wykluczającymi z udziału w badaniu były: 1) niekontrolowana cukrzyc (glikemia na czczo lub glikemia przygodna w trakcie hospitalizacji przekraczająca odpowiednio 140 mg% lub 200 mg% albo HbA1c > 7,5%), 2) niekontrolowane nadcisnienie tętnicze (> 160/90 mmHg w więcej niż 50% pomiarów w ciągu poprzedzającego miesiąca); 3) przewlekta niewydolność krążenia w klasie wg NYHA wyższej niż I lub/i frakcja wyrzutowa lewej komory w aktualnym badaniu echokardiograficznym < 40%; 4) hemodynamicznie istotna zastawkowa wada serca; 5) ostre zakażenie w ciągu poprzedzających 3 tygodni; 6) przewlekta choroba zapalna (m.in. przewlekta obturacyjna choroba płuc – PochP, choroby reumatologiczne); 7) niedokrwistość (Hb < 11 g%); 8) choroba nowotworowa; 9) udar niedokrwienny w wywiadzie lub inne zespoły niedokrwienne, poza ChW.

 Wyniki: Stężenie Hsp27 w surowicy krwi obwodowej pacjentów z istotną ChW wykazywało jedynie tendencję do wyższych wartości w porównaniu ze stężeniem w krwi osób z prawidłowymi tętnicami wieńcowymi [0,463 (0,158–0,809) vs 0,184 (0,099–0,337) ng/ml, odpowiednio; p = 0,084]. Stężenie Hsp27 było natomiast wyższe w surowicy pacjentów z 2- i 3-naczyniową ChW [0,529 (0,192–1,004) ng/ml] w porównaniu z osobami bez zmian w tętnicach wieńcowych (p = 0,035) i w porównaniu z pacjentami z jedno-naczyniową ChW [0,276 (0,087–0,549) ng/ml, p = 0,041]. Stężenie Hsp27 w surowicy nie wykazywało korelacji z ciężkością ChW ocenianą na podstawie skali Gensiniego (r = 0,21, p = 0,11).

Wnioski: Stężenie Hsp27 w surowicy krwi obwodowej może świadczyć o rozległym niedokrwieniu miokardium i zaawansowanej ChW.

Słowa kluczowe: choroba wieńcowa, miażdżyca tętnic wieńcowych, rezerwa przepływu wieńcowego

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