Increased Myocardial Apoptosis in Patients With Unfavorable Left Ventricular Remodeling and Early Symptomatic Post-Infarction Heart Failure

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OBJECTIVES

The purpose of this study was to evaluate a potential correlation between apoptotic rate (AR), post-infarction left ventricular (LV) remodeling, and clinical characteristics in subjects who died late (≥10 days) after an acute myocardial infarction (AMI) with evidence of persistent occlusion of the infarct-related artery at autopsy.

BACKGROUND

Apoptosis contributes to myocardocyte loss in cardiac disease and may have a pathophysiologic role in post-infarction LV remodeling.

METHODS

The AR was calculated at the site of infarction and in remote unaffected LV regions, using co-localization of in situ end labeling for deoxyribonucleic acid fragmentation and immunohistochemistry for caspase-3, in 14 subjects who died within two months after AMI. Correlation between AR and clinical characteristics such as age, site of AMI, transmural extension, multivessel coronary disease, and signs and/or symptoms of heart failure (HF), at the time of initial hospitalization for AMI or subsequently before death, was assessed using non-parametric statistical tests. Parameters of LV remodeling including diameters, free wall thickness, diameter-to-wall-thickness ratio, and mass were measured at gross examination at autopsy. Values are expressed as median (interquartile range).

RESULTS

Among clinical variables, early symptomatic post-infarction HF (9 cases, 64%) was associated with nearly fourfold increased AR at the site of infarction (26.2% [24.5% to 28.8%] vs. 6.4% [1.9% to 13.3%], p = 0.001). Moreover, AR both at the site of infarction and in unaffected regions was significantly correlated with parameters of progressive LV remodeling (p < 0.05).

CONCLUSIONS

Our data show that in patients dying ≥10 days after AMI, myocardial apoptosis is strongly associated with and may be a major determinant of unfavorable LV remodeling and early symptomatic post-infarction HF. (J Am Coll Cardiol 2003;41:753–60) © 2003 by the American College of Cardiology Foundation
correlation between AR at the time of death (both at the site of infarction and in unaffected LV areas), clinical characteristics of the subjects in the days preceding death (including the occurrence of signs or symptoms of HF), and macroscopic signs of LV remodeling in subjects dying late (10 to 62 days) after AMI, all with persistent occlusion of the infarct-related artery (IRA) occlusion (as IRA status may itself influence AR) (16).

METHODS

We selected, at routine postmortem examination, 14 subjects according to the following inclusion criteria: 1) death due to nontraumatic cause, occurring 10 to 62 days after an AMI; 2) persistent occlusion of the IRA documented at postmortem examination; and 3) no clinical or pathologic evidence of HF. We collected data on initial hospitalization for AMI in all cases. Site of infarction, IRA, initial treatment (including fibrinolytic treatment for ST-segment elevation AMI), and past medical history were obtained from clinical charts. Clinical and laboratory data regarding the days preceding death were available in all cases. Re-infarction was excluded on the basis of clinical, laboratory, and pathology data. Patients were considered to be suffering from symptomatic HF if one or more of the following signs, symptoms, or clinical characteristics had been present in the days before death: dyspnea at rest (not due to concomitant pulmonary or musculoskeletal disorders) associated with jugular venous distension, third heart sound and/or pulmonary rales, peripheral edema, elevated pulmonary wedge pressure and/or central venous pressure, reduced cardiac output, need of intravenous inotropic support and significant (>4.5 kg) decrease in weight after diuretic use (Stages C or D and New York Heart Association functional class IV according to American College of Cardiology/American Heart Association guidelines for the evaluation and management of HF [17]). Treatment by insulin or oral antidiabetic agents was used to define diabetics.

Pathology. Gross examination of the hearts was performed to measure various LV parameters, to define the infarcted area, and to confirm total IRA occlusion. Cardiac diameters were calculated at the atrioventricular section, and LV free wall thickness was measured at the median third of the unaffected free wall (usually the posterior wall). Infarcts were defined as large infarcts if an area of transmural necrosis involving more than one LV wall (approximately 30% of the circumference) was found at pathology. Left ventricular hypertrophy was defined as LV wall thickness ≥15 mm in unaffected LV regions. A transverse diameter-to-free wall thickness ≥9 was used to define LV dilation. The combination of hypertrophy and dilation was defined as eccentric hypertrophy. Pulmonary, liver, and/or renal congestion and signs of renal and/or intestinal hypoperfusion were considered pathologic evidence of HF as indirect signs of chronic congestive state and circulatory failure (4,18).

Tissue specimens were obtained at sites of infarction (Fig. 1A) and in unaffected regions of the LV remote from the infarcted area. Specimens were fixed in 10% paraformaldehyde. In situ end labeling of deoxyribonucleic acid (DNA) fragmentation terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) was performed using the Apoptag kit (Oncor, Gaithersburg, Maryland), according to the supplier’s instructions (Fig. 1B). For immunohistochemistry, the sections already treated for the TUNEL assay were heated and then incubated with antibodies against muscle actin (mouse monoclonal anti-human actin HHF35 from Dako, Carpinteria, California; dilution 1:50) and caspase-3 (rabbit polyclonal anti-human caspase-3 from Upstate Biotec technology, Lake Placid, New York; dilution 1:100) and visualized by the streptavidin–biotin system (Dako), using either 3-amino-9-ethycarbazide (AEC) or diaminobenzidine as the final chromogen (Figs. 1C and 1D). Myocardocytes were defined as apoptotic if co-localization of markers of DNA fragmentation (TUNEL) and caspase-3 was evident (Fig. 1D), according to the fact that high immunohistochemical expression of caspase-3 detected with the antibody used in this study is present in myocardocytes undergoing apoptosis, co-localizes with TUNEL-positive myocardiocytes, and corresponds mostly to increased expression of its activated form (9,19,20). The AR was expressed as the ratio of the number of myocardocytes co-expressing TUNEL and caspase-3 positivity on nucleated cells per field (250×). Muscle actin-negative cells as well as myocardocytes co-expressing TUNEL positivity and specific staining for markers of DNA synthesis (proliferating cell nuclear antigen [PCNA]) (using mouse monoclonal anti-human PCNA PC10 antibody from Dako, dilution 1:100) and/or markers of transcription activity (ribonucleic acid splicing factor SC-35) (using mouse monoclonal anti SC-35 from Sigma, Milan, Italy; dilution 1:200) were not included in the cell count. TUNEL-negative/caspase-3-positive cells, although likely to be cells committed to apoptosis, were not considered to be apoptotic because caspase-3 activation may represent a reversible step in the apoptotic cascade (6). Suitable negative and positive controls for TUNEL and caspase-3 were performed, as defined elsewhere (9). Briefly, controls for TUNEL were performed as indicated by the
supplier (using a normal female rodent mammary gland 3 to 5 days after weaning of rat pups for positive control and sham staining leaving out active terminal deoxynucleotidyl transferase but including proteinase K digestion to control for nonspecific incorporation of nucleotides or for nonspecific binding of enzyme-conjugate). A human lymph node was used as a control for activated caspase-3 (strong immunoreactivity was evident in the apoptotic-prone germinal center B-lymphocytes of the lymph node and not in the mantle zone). Moreover, negative controls indicating the non-interference of TUNEL and secondary antibodies were performed by leaving out the primary antibodies (actin, caspase-3, PCNA and SC-35 respectively).

**Statistical analysis.** For statistical analysis, the software SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois) was used. Quantitative results were expressed as median (interquartile range). Nonparametric tests for non-paired data were used to compare AR among different subjects, the Mann-Whitney U test was used when two groups were compared, and the Kruskal-Wallis test was used when more than two groups were compared. Bonferroni’s correction was applied to the Mann-Whitney U test when comparisons between multiple groups were performed. Correlation between continuous variables was calculated with the Spearman correlation test. Discrete variables were compared by logistic regression analysis. For variables associated with statistical significance or borderline significance (p < 0.10), multivariable analysis was performed using a generalized linear model following a multiple linear regression model (using the default identity link function available on SPSS 10.0).

**Table 1.** Clinical and Demographic Characteristics of the Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients</th>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>14</td>
</tr>
<tr>
<td>Age (median, yrs)</td>
<td>74</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>(69–79)</td>
</tr>
<tr>
<td>Gender (males, %)</td>
<td>11 (79%)</td>
</tr>
<tr>
<td>Clinical characteristics (%)</td>
<td></td>
</tr>
<tr>
<td>Anterior AMI</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Transmural AMI</td>
<td>12 (86%)</td>
</tr>
<tr>
<td>Large AMI</td>
<td>10 (71%)</td>
</tr>
<tr>
<td>Fibrinolytic treatment at time of initial AMI</td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Persistent IRA occlusion</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Multivessel coronary disease</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Previous remote AMI (≥6 months earlier)</td>
<td>7 (50%)</td>
</tr>
<tr>
<td>Symptomatic HF</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Time from AMI to death (median, days)</td>
<td>16 (12–34)</td>
</tr>
</tbody>
</table>

AMI = acute myocardial infarction; HF = heart failure; IRA = infarct-related artery.
had nearly fourfold increased AR versus the others (26.2% [24.5% to 28.8%] vs. 6.4% [1.9% to 13.3%], p = 0.001) (Fig. 2). Fibrinolysis at the time of AMI (4 cases) and a history of previous remote AMI (≥6 months earlier than the more recent AMI; 7 subjects) were associated with a nonsignificant trend toward higher AR (28.8% [20.5% to 32.8%] vs. 19.4% [5.6% to 26.0%], p = 0.054 and 28.4% [17.9% to 29.1%] vs. 14.6% [3.2% to 25.9%], p = 0.073 for fibrinolysis and previous remote AMI, respectively). At multivariable analysis including these three parameters, however, only HF remained significantly associated with increased apoptosis (p < 0.001), whereas effects of fibrinolytic treatment were lost (p = 0.956) as well as history of previous remote AMI (p = 0.476).

When compared with the others, subjects with signs or symptoms due to HF before death had, as expected, greater LV transverse diameter (137 vs. 120 mm, p = 0.012), longitudinal diameter (110 vs. 100 mm, p = 0.029), transverse diameter-to-LV free wall thickness (10.0 vs. 8.0, p = 0.029), smaller LV free wall thickness (12 vs. 15 mm, p = 0.08), and greater cardiac mass (530 vs. 460 g, p = 0.029) at autopsy. Moreover, they were less likely to have concentric compensatory hypertrophy (0% vs. 60%, p = 0.027), and more frequently they had indirect signs of systemic congestion and/or hypoperfusion compatible with the effects of a failing circulation assessed at pathology (80% vs. 40%, p = 0.25). Interestingly, considering the subjects who met the clinical and/or pathologic criteria to define HF, they (11 cases) had a significantly higher AR versus the others, both at the site of infarction (25.9% [17.9% to 28.6%] vs. 6.4% [0.5% to 12.0%], p = 0.022) and in remote regions (0.7% [0.7% to 0.9%] vs. 0.3% [0.3 to 0.4], p = 0.022).

Apoptosis and cardiac remodeling. Analysis of correlation between apoptosis and macroscopic signs of LV remodeling revealed a statistically significant link between AR at both sites (infarction and remote unaffected site) and macroscopic signs of cardiac remodeling (LV transverse and longitudinal diameters, free wall thickness, diameter-to-wall thickness ratio, and mass) (Table 2, Fig. 3).

Left ventricular dilation was found in 10 cases (71%), and it was associated with significantly higher AR at the site of infarction (26.0% [22.6% to 28.7%] vs. 7.6% [1.2% to

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**Table 2.** Gross Anatomy Characteristics of the Hearts and Correlation With Apoptotic Rates at Site of Infarction and in Unaffected LV Sites

<table>
<thead>
<tr>
<th>Cardiac diameters (mm)</th>
<th>Infarction Site</th>
<th>Remote Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Transverse</td>
<td>0.72</td>
<td>0.004*</td>
</tr>
<tr>
<td>Longitudinal</td>
<td>0.66</td>
<td>0.011*</td>
</tr>
<tr>
<td>LV wall thickness (mm)</td>
<td>-0.52</td>
<td>0.050*</td>
</tr>
<tr>
<td>Diameter-to-thickness ratio</td>
<td>0.63</td>
<td>0.015*</td>
</tr>
<tr>
<td>Cardiac weight (g)</td>
<td>0.48</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* p < 0.05.

LV = left ventricular.
Figure 3. Correlations between macroscopic signs of post-infarction left ventricular (LV) remodeling and apoptotic rate (AR) at the site of acute myocardial infarction and in remote unaffected LV regions. Correlation between AR at the site of infarction and LV longitudinal (A) and transverse (B) diameter, free wall thickness (C), transverse diameter-to-free wall thickness (D), and cardiac weight (E). Correlation between AR in remote regions and the same LV macroscopic parameters (F to L), respectively. Black and white dots represent subjects with (filled squares) and without (empty squares) early symptomatic post-infarction heart failure, respectively.
Six subjects (43%) had signs of LV hypertrophy. The AR in hearts with LV hypertrophy was significantly reduced in the surviving unaffected areas (0.4% [0.3% to 0.7%] vs. 0.9% [0.7% to 1.0%], p = 0.029). The combined effect of LV dilation and hypertrophy is shown in Figure 4. The AR in both infarct and remote sites progressively increased comparing hearts with concentric compensatory hypertrophy (early stage of LV remodeling) (3 cases), decompensated eccentric hypertrophy (hypertrophy and dilatation, transition stage) (3 cases), and eventual ventricular dilation with wall thinning (end stage) (7 cases) (Fig. 4).

DISCUSSION

This study not only confirms previous evidence of the presence of myocardiocyte apoptosis late after AMI (9–11,13–15) but also shows that apoptosis, both at the site of infarction and in unaffected sites of the LV, correlates with unfavorable post-infarction LV remodeling and the occurrence of HF after AMI. In fact, patients with enhanced myocardial apoptosis at the site of infarction at the time of death were significantly more likely to have hearts with signs of unfavorable remodeling and to have suffered from symptoms or signs of HF early after AMI.

Saraste et al. (21) have shown that patients with more severe HF and rapid clinical progression had higher AR at the time of transplantation versus patients with slower progression. This correlation between AR and disease progression in dilated cardiomyopathy has been recently prospectively validated in a study by Metzger et al. (22) in patients undergoing partial ventriculectomy.

The association between apoptosis and unfavorable prognosis may be, at least in part, explained by a causal role of myocardiocyte apoptosis in the pathophysiology of progressive cardiac remodeling leading to cardiac and circulatory failure. Indeed, there is in our data a statistically relevant correlation between AR and gross parameters of LV remodeling, as LV cavity size. The latter is considered a strong independent clinical prognostic factor (23) and a potential surrogate end point for prognosis in patients with HF (24).

Moreover, AR correlated not only with macroscopic signs assessed at pathology but also with the signs and symptoms of decompensated HF before death. Interestingly, the occurrence of these features does not appear irrelevant in the clinical assessment of patients with HF, because it is associated with increased mortality independently from other clinical variables (such as LV ejection fraction) (25–28). Although speculative, it is possible that enhanced apoptosis may be responsible for a more rapid disease progression in patients showing unfavorable cardiac remodeling and symptomatic HF. It has been suggested that the peripheral circulation, more than cardiac performance, is responsible for the development of symptoms (29). In fact, it is difficult from morphologic examination of the heart to differentiate a damaged but compensated heart from one that is failing. This may suggest that peripheral neurohormonal rearrangements may be associated with more pronounced clinical presentation and may be responsible, at least in part, for progressive deterioration of HF (30).

Initial phases of cardiac remodeling are often characterized by compensatory cardiac and non-cardiac mechanisms. Left ventricular hypertrophy of the surviving portions of the heart is considered an initially effective compensatory phenomenon (3), characterized by a low-grade apoptosis and a more favorable prognosis when compared with LV dilation.
Apoptosis may play a role in the transition from compensated concentric hypertrophy to decompensated eccentric hypertrophy (31–33).

Increased plasma norepinephrine levels are part of the peripheral neurohormonal rearrangements (30). Interestingly, they are associated with a worse prognosis (34), whereas treatment with beta-blockers favorably affects prognosis in patients with HF (35). Of note, beta1-receptor agonism is associated with myocardiacyte apoptosis in vitro (36), and treatment with metoprolol significantly reduces myocardiacyte apoptosis in an experimental HF model in dogs (15). The same effects appear to be exerted by angiotensin II (34,37), whereas angiotensin-converting enzyme inhibitors modulate the unfavorable neurohormonal rearrangements (34), cardiac remodeling (38), and apoptosis (14) and improve symptoms and prognosis in HF.

Therefore, two forms of apoptosis appear to affect the course of post-infarction remodeling, both leading to unfavorable LV remodeling and symptomatic HF: ischemia-driven apoptosis at the site of recent infarction (7–10,13–15,21), and load-dependent or receptor-dependent apoptosis at sites remote from the ischemic area (11,32,33,36,37,39). An early article by Gottlieb et al. (40), being the first study to evaluate apoptosis in the ischemic heart, reported an increased AR in an ischemia–reperfusion model, suggesting that apoptosis was associated with reperfusion more than with hypoxia alone and supporting the concept that completion of the apoptotic cascade depends on the available energetic levels (6). However, subsequent studies have completed this scenario by showing that, although reperfusion after myocardial ischemia accelerates apoptosis in the non–salvageable myocardiocytes, reperfusion is also associated with a significantly lower total number of cells undergoing apoptosis, supporting an overall beneficial effect by reperfusion (41). Caspase-3 activation represents one of the terminal phases of the apoptotic cascade, thus representing a potential target for anti-apoptotic therapy. Although initial experimental studies using caspase inhibitors in animal models showed interesting results, clinical trials using such inhibitors are still ongoing (42).

Most studies investigating apoptosis in vivo or ex vivo, including the present study, however, suffer from the major drawback of selection bias. Indeed, the evaluation of subjects dying 10 to 62 days after AMI may have led to the selection of a group of patients with more adverse prognosis and extremely elevated AR, in comparison to the great majority of cases surviving several weeks and months after AMI. These considerations may explain the differences in AR in our samples when compared with animal models or with models of end-stage HF in patients undergoing cardiac transplantation, even if both of these models may suffer from inverse selection bias (6). As discussed by Sam et al. (11), the evaluation in animal models of only those individuals surviving until the established temporal end point for sacrifice may have led to underestimation of AR, as nearly 65% of the animals died spontaneously beforehand and therefore were not analyzed (11). Similarly, analysis of the hearts explanted from patients undergoing cardiac transplantation (12,21) may have not been able to assess AR in those patients with more severe HF who did not survive long enough to receive a cardiac transplant. In vivo validation of our data will therefore be necessary, and it would be extremely useful also to assess whether patients with increased myocardial apoptosis may be identified early through determination of clinical characteristics (such as signs of HF), of levels of plasma markers (such as soluble tumor necrosis factor receptors [43]), and/or of apoptosis-targeted molecular imaging such as myocardial perfusion scan using marked annexin V (44).

Conclusions. Results in our data show that in patients with AMI dying 10 to 62 days after the infarction with persistent IRA occlusion at the time of death, apoptosis is strongly associated with, and may be a major determinant for developing, unfavorable LV remodeling and early symptomatic HF. A direct cause–effect link, however, cannot be assumed by our data, and further studies are required to define the precise mechanisms and impact of apoptosis on ischemic LV dysfunction, as well as potential applications in the development of improved diagnostic and therapeutic measures. Given the small sample size, the borderline statistical significance in some of the analyses performed, the possible confounding factors, and the unknown duration of the apoptotic cascade in human myocardiocytes, mechanistic and causal connections should be cautiously drawn.

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