Right ventricular dilatation after left ventricular acute myocardial infarction is predictive of extremely high peri-infarctual apoptosis at postmortem examination in humans


Background: Cardiac remodelling after acute myocardial infarction (AMI) is characterised by molecular and cellular mechanisms involving both left and right ventricles, and biventricular failure identifies patients with an extremely unfavourable prognosis.

Aims: To assess whether a link exists between increased myocardial apoptotic rates (AR) at sites of recent infarction and patterns of unfavourable cardiac remodelling, such as biventricular enlargement after left ventricular (LV) infarction.

Methods: Twelve patients with recent AMI involving the LV and not the right ventricle (RV) and with permanent infarct related artery occlusion were selected at necropsy. Gross pathological characteristics, such as LV and RV dilatation, and AR at site of infarction were assessed. Potential false positive results (DNA synthesis and RNA splicing) were excluded from the cell count.

Results: RV enlargement, defined as a tricuspidal ring greater than 120 mm, was found in five cases and was associated with LV dilatation. These patients showed significantly higher AR than the others. When the subjects were divided into three groups according to progressive cardiac remodelling (absence of cardiac dilatation, isolated LV dilatation, and biventricular enlargement), the last group had significantly higher ARs than the other two groups, showing that myocardioocyte apoptosis is increased in more unfavourable forms of cardiac remodelling.

Conclusion: Patients with severely unfavourable cardiac remodelling, such as biventricular enlargement, have extremely high myocardioocyte apoptosis at necropsy, even late after LV myocardial infarction, supporting the role of myocardioocyte loss in determining post-infarction adverse remodelling.

Acute myocardial infarction (AMI) is associated with early and late compensatory mechanisms in the attempt to optimise ventricular filling and cardiac output. The greater the initial myocardial insult the greater the remodelling response and less favourable the longterm prognosis. In experimental models of AMI in animals, right ventricular (RV) remodelling is associated with left ventricular (LV) remodelling, even if the RV is spared from the initial ischaemic damage. 1–3 Hirose et al have shown that RV dilatation and remodelling variably occur in subjects with transmural LV myocardial infarction. 1 Biventricular dilatation is more pronounced in large AMI and is often associated with pulmonary congestion. The presence of signs and/or symptoms resulting from RV failure identifies a subgroup of patients with an extremely unfavourable prognosis, and survival is usually less than two years. 4–5

“It is still unclear whether a link exists between increased apoptotic rates and extremely unfavourable forms of cardiac remodelling, such as that characterised by biventricular enlargement.”

Several molecular and cellular mechanisms, occurring both at the site of infarction and in remote unaffected sites, lead to cardiac enlargement and dysfunction after AMI. 6–7 Recently, myocardioocyte loss as a result of apoptosis in early and subacute phases of AMI has been consistently shown to be present in human observational studies and in experimental animal models, and increased myocardial apoptotic rates (AR) are associated with severe and progressive heart failure (HF). 8, 9 However, it is still unclear whether a link exists between increased AR and extremely unfavourable forms of cardiac remodelling, such as that characterised by biventricular enlargement. To investigate this issue, the extent of post-infarction myocardial apoptosis was assessed at postmortem examination in a cohort of subjects who died within two months of AMI, and who had a variable degree of cardiac remodelling.

METHODS

Twelve white subjects were selected at postmortem examination according to the following inclusion criteria: (1) death occurring 10 days to two months after an AMI involving only the LV and not the RV; (2) no clinical or pathological evidence of reinfarction; (3) persistent infarct related artery occlusion (IRA) at postmortem examination; and (4) absence of conditions likely to affect RV remodelling (such as primary lung disease, pulmonary embolism, pulmonary valvulopathy, and intracardiac shunts). Initial treatment and past and recent

Abbreviations: AMI, acute myocardial infarction; AR, apoptotic rates; HF, heart failure; IRA, infarct related artery occlusion; LV, left ventricular; PCNA, proliferating cell nuclear antigen; RV, right ventricular; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase mediated dUTP nick end labelling.
immunohistochemical expression of caspase 3 is present in cardiomyocytes undergoing apoptosis and colocalises with TUNEL positive cardiomyocytes. The AR was expressed as the ratio of the number of cardiomyocytes showing both TUNEL and activated caspase 3 positivity out of the total number of nucleated cells in each field (magnification, ×250), calculated after counting 100 fields. Muscle actin negative cells, in addition to cardiomyocytes showing both TUNEL positivity and specific staining for markers of DNA synthesis (proliferating cell nuclear antigen [PCNA]; using mouse monoclonal anti-human PCNA PAb10 antibody; Dako; dilution, 1/100) and/or markers of transcription activity (RNA splicing factor SC-35; using mouse monoclonal anti-SC-35; dilution, 1/200 Sigma, Milan, Italy) were not included in the cell count, because they were considered potential false positive results.

**Pathology**

Gross examination of the hearts was performed to measure cardiac parameters and to define the infarcted area and the IRA. Infarct areas were classified as transmural or non-transmural infarcts and as small or large (involving more than one LV wall) infarcts. Permanent IRA occlusion at time of death was defined as a complete absence of residual lumen at pathological examination as a result of atheroma and/or thrombosis. 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). A transverse LV diameter greater than 90 mm and/or a cardiac diameter to free wall thickness ratio > 9 in the absence of RV enlargement were used to define LV dilatation. RV dilatation was defined as an enlargement of the right ventricle, characterised by a tricuspidal ring circumference greater than 120 mm.

Tissue specimens were obtained at sites of infarction and in remote unaffected LV free wall (usually posterior wall), and they were processed as described previously. Briefly, specimens were fixed in 10% paraformaldehyde, terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick end labelling (TUNEL) was performed using the ApopTag kit (Oncor, Gaithersburg, Maryland, USA), according to the supplier’s instructions. 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; dilution, 1/50; Dako, Carpenteria, California, USA) and activated caspase 3 (anti-cleaved caspase 3 (Asp 175) antibody; dilution, 1/50; Cell Signaling Technology, Piscataway, New Jersey, USA) and visualised by means of the streptavidin–biotin system (Dako), using either 3-amino-9-ethylcarbazide or diaminobenzidine as the final chromogen. Streptavidin–biotin system (Dako), using either 3-amino-9-ethylcarbazide or diaminobenzidine as the final chromogen. Multifocal coronary disease

Signs and symptoms of HF

Previous additional AMI

LV dilatation

Cardiac weight in grams (interquartile range)

Median Norris index (interquartile range)

Median time from AMI to death in days (interquartile range)

<table>
<thead>
<tr>
<th>Clinical and demographic characteristics of the patients</th>
<th>RV dilatation</th>
<th>No RV dilatation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Median age in years (interquartile range)</td>
<td>78 (76–80)</td>
<td>71* (63–74)</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Clinical features</td>
<td></td>
<td></td>
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<tr>
<td>Transmural AMI</td>
<td>4</td>
<td>7</td>
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<td>Septal AMI</td>
<td>2</td>
<td>5</td>
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<tr>
<td>Anterior AMI</td>
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<td>4</td>
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<tr>
<td>Large AMI</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Multifocal coronary disease</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Signs and symptoms of HF</td>
<td>5</td>
<td>2*</td>
</tr>
<tr>
<td>Previous additional AMI</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>LV dilatation</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac weight in grams (interquartile range)</td>
<td>530 (460–625)</td>
<td>490 (467–545)</td>
</tr>
<tr>
<td>Median Norris index (interquartile range)</td>
<td>12 (10–13)</td>
<td>6 (4–13)</td>
</tr>
<tr>
<td>Median time from AMI to death in days (interquartile range)</td>
<td>25 (18–44)</td>
<td>16 (14–23)</td>
</tr>
</tbody>
</table>

*p<0.05.

AMI, acute myocardial infarction; HF, heart failure; LV, left ventricular; RV, right ventricular.

Statistical analysis

The software SPSS 10.0 for Windows (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Quantitative results are expressed as median (interquartile range). The non-parametric Mann-Whitney U test and the Kruskal-Wallis test for non-paired data were used to compare AR values among different subjects, when comparing two or more than two groups, respectively. The χ² test was used to compare discrete variables, and Fisher’s exact test was used when one or more cells contained a value lower than 5. Logarithmic transformation was used to perform the post hoc test for linear trend at ANOVA univariate analysis.
RESULTS

Clinical data

Table 1 shows the clinical characteristics of the patients. The median time to death after AMI was 20 days. Seven patients had been given a diagnosis of HF at the time of initial hospitalisation for AMI or subsequently before death. All but one subject had had a transmural infarct, six of which involved the anterior wall and/or interventricular septum. Large infarcts were present in nine of 12 patients. Five patients had large transmural anterior and septal AMI. The median Norris coronary prognostic index calculated at the time of admission was 10 (interquartile range, 4–13), indicating a high risk clinical profile in this population.

Cardiac remodelling

Table 1 summarises the gross anatomical characteristics of the hearts. Nine patients had LV dilatation, which was isolated in four patients but combined with RV dilatation in five (fig 1). No patients had isolated RV dilatation. Subjects with RV dilatation had similar clinical characteristics to the other patients (table 1), although they were significantly older (78 v 71 years old; p = 0.030) and more frequently had had symptoms or signs of HF (five of five v two of seven; p = 0.027). Interestingly, although all patients with biventricular remodelling had had symptoms and/or signs of HF, only half of the subjects with isolated LV dilatation and none of those with more favourable cardiac remodelling and undilated hearts had had a diagnosis of HF (p = 0.019). Moreover, the Norris index was significantly different between the three groups (4 v 9 v 12; p = 0.040).

Myocardial apoptosis in RV dilatation

Gross pathological and standard microscopic examination showed that scar tissue consistent with previous necrotic cell death was evident in all patients, in the absence of signs of very recent or acute ongoing necrosis. Indeed, the presence of ongoing necrosis was an exclusion criterion because it may be a confounding factor when assessing the role of apoptosis. TUNEL and immunostaining for activated caspase 3 colocalised in more than 90% of cells. Only double positive myocardiocytes were considered apoptotic. A median of 21% of myocardiocytes were apoptotic at sites of recent infarction and 0.7% in remote regions of the LV. Intense immunostaining for activated caspase 3 was evident at sites of infarction (including some TUNEL negative cells) but not in remote regions, where few myocardiocytes were considered positive. A comparison of immunostaining for both non-activated (rabbit polyclonal antihuman caspase 3; dilution, 1/10019; Upstate Biotechnology, Lake Placid, New York, USA) and activated caspase 3 showed almost identical results, supporting the hypothesis that caspase 3 is both overexpressed and subsequently activated at sites of infarction (fig 2).

**Figure 1**  Left ventricular and biventricular enlargement. (A) A case of recent posterior wall acute myocardial infarction (day 17) complicated by mild left ventricular dilatation. (B) A case of severe biventricular remodelling following anterior wall acute myocardial infarction (day 15).

**Figure 2**  Demonstration of apoptosis. (A) Labelling of nuclear DNA fragmentation using terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL). Colocalisation of (B) TUNEL and actin and of (C) TUNEL and activated caspase 3.

**Figure 3**  Myocardial apoptosis and right ventricular (RV) dilatation. Subjects with RV remodelling after left ventricular acute myocardial infarction have a significantly higher apoptotic rate at the site of recent infarction compared with the other groups. The columns represent the median value and the vertical bars show the interquartile range.
groups (3%–14%) vs 10% (3–18%); p = 0.048; fig 3). When subjects were divided into three groups (non-dilated hearts, isolated LV dilatation, and combined LV and RV dilatation), there was a significant difference in AR among the three groups (3% vs 14% vs 26%, respectively; p = 0.050; figs 4, 5). Logarithmic transformation was also performed in case of potential deviations from normality and linearity. This confirmed the association between the three different groups and increasing AR in ANOVA univariate analysis (p = 0.023), showing a p value for trend of 0.008 in the post hoc test for linear trend (fig 5). No significant differences were found when comparing AR values at remote LV sites between the three different groups.

**DISCUSSION**

Post-infarction cardiac remodelling leading to HF represents a “dangerous intersection” in AMI patients, and biventricular failure is considered to be the terminal stage of cardiac remodelling. Our study shows for the first time that this high risk condition is associated with extremely high peri-infarct AR at postmortem examination, confirming and greatly expanding previous findings of a correlation between myocardial apoptosis and unfavourable LV remodelling. Although speculative, it is possible that the extremely unfavourable prognosis associated with biventricular remodelling (supported in our data by the raised Norris index and the presence of signs of HF in all patients) might be the result not only of greater infarct sizes but also of increased myocardial apoptosis. Interestingly, an association between high risk clinical features with a relevant prognostic value, as computed in the Norris index, and increased AR has been reported recently.

Right sided HF is mainly characterised by a low cardiac output state and systemic congestion. Neurohormonal rearrangements (such as adrenergic hyperactivation and activation of the renin–angiotensin system) are particularly evident in this clinical feature, and may not only represent an epiphemomenon but may also have a direct pathophysiological role. Sabbah et al have shown that multiple myocardial infarctions in dogs lead to biventricular remodelling and increased mortality rates. The same authors have subsequently reported significantly increased AR values in the hearts of these animals (mainly in peri-infarct regions), and the potential modulation by angiotensin converting enzyme inhibitors and β blockers.

“Our results support the role of progressive myocardio-cyte loss in determining unfavourable remodelling”

However, it is still unclear exactly how AMI of the LV affects remodelling of the RV. Pulmonary hypertension and secondary increases of the RV afterload are thought to be the main pathophysiological mechanisms, although other hypotheses should also be considered. Abnormal interventricular septum contractility, concomitant RV ischaemia, and afterload increase resulting from ischaemic mitral insufficiency may be responsible for additional RV strain. Interestingly, in our analysis, which was limited by the small sample size, no significant association was found between RV dilatation and the site of AMI or multivessel coronary artery disease. Taken together, our results identify the major determinant for the development of biventricular failure as the extent of myocardio-cyte loss at the site of infarction. Among established and potential modulators of myocardial apoptosis, age, sex, ischaemia, infarct related artery occlusion, HF, and cardiac dilatation have all been shown to be predictors of increased apoptosis. Both increased wall stress (as a result of LV failure) and the enhanced production of angiotensin II and of catecholamines (as a result of HF) may further stimulate apoptosis in the ischaemic peri-infarctual myocardium, and may also cause increased apoptosis in areas of the myocardium remote from the infarction site, both in the LV and RV.
Take home messages

- Patients with severely unfavourable cardiac remodelling, such as biventricular enlargement, have extremely high myocardicocyte apoptosis even at a late time after LV myocardial infarction. Our results support the role of progressive myocardicocyte loss in determining unfavourable remodelling. However, as with all observational studies, our study may be fraught with potential limitations, such as unexplained selection biases. Moreover, the incomplete definition of the temporal and spatial burden of increased apoptosis is a limitation when interpreting these results. The extremely high AR (up to 25%) at the site of infarction may seem difficult to interpret. However, the following should be kept in mind: (1) this high rate was confined to the surviving myocardium at the infarct zone; (2) selection criteria included only cases with extremely unfavourable prognosis, enrolled at postmortem examination; (3) this persistent myocardicocyte loss may be limited in time following AMI; (4) all cases had persistent infarct related artery occlusion, which is known to be associated with increased AR(10); and (5) the data presented agree with previous published studies enrolling individuals with similar clinical characteristics. Moreover, it is possible that jeopardised myocardium (where the apoptotic cascade is already partially activated, but kept within the boundary of reversibility) may be detrimentally affected by perimortem events, leading to significantly higher rates of cell death at postmortem examination. [AQ:5]

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