Atrial natriuretic peptide, B-type natriuretic peptide, and serum collagen markers after acute myocardial infarction

Jarkko Magga,1 Mikko Puhakka,1 Seppo Hietakorpi,1 Kari Punningen,2 Paavo Uusimaa,3 Juha Risteli,2,4 Olli Vuolteenaho,5 Heikki Ruskoaho,6 and Keijo Peuhkurinen1

Departments of 1Internal Medicine and 2Clinical Chemistry, Kuopio University Hospital, 70211 Kuopio; Departments of 3Internal Medicine and 4Clinical Chemistry, Oulu University Hospital, and Departments of 5Physiology and 6Pharmacology and Toxicology, University of Oulu, 90014 Oulu, Finland

Submitted 27 May 2003; accepted in final form 5 November 2003

Magga, Jarkko, Mikko Puhakka, Seppo Hietakorpi, Kari Punkkonen, Paavo Uusimaa, Juha Risteli, Olli Vuolteenaho, Heikki Ruskoaho, and Keijo Peuhkurinen. Atrial natriuretic peptide, B-type natriuretic peptide, and serum collagen markers after acute myocardial infarction. J Appl Physiol 96: 1306–1311, 2004. First published November 7, 2003; 10.1152/japplphysiol.00557.2003.—Experimental data suggest that atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) act locally as antifibrotic factors in heart. We investigated the interrelationships of natriuretic peptides and collagen markers in 93 patients receiving thrombolytic treatment for their first acute myocardial infarction (AMI). Collagen formation following AMI, evaluated as serum levels of amino terminal propeptide of type III procollagen, correlated with NT-proANP (r = 0.45, P < 0.001), BNP (r = 0.55, P < 0.001) and NT-proBNP (r = 0.50, P < 0.01) on day 4 after thrombolysis. Levels of intact amino terminal propeptide of type I procollagen decreased by 34% (P < 0.001), and levels of carboxy terminal cross-linked telopeptide of type I collagen (ICTP) increased by 65% (P < 0.001). ICTP levels correlated with NT-pro-terminal proBNP (r = 0.25, P < 0.05) and BNP (r = 0.28, P < 0.05) on day 4. Our results suggest that ANP and BNP may act as regulators of collagen scar formation and left ventricular remodeling after AMI in humans. Furthermore, degradation of type I collagen is increased after AMI and may be regulated by BNP.

INTRODUCTION

ATRIAL NATRIURETIC PEPTIDE (ANP) and B-type natriuretic peptide (BNP) are natriuretic, diuretic, and vasodilating hormones that are secreted from the heart in response to mechanical stress (16, 36). ANP is stored as a 126-amino acid peptide proANP (38). Cleavage of proANP results in the release of NH₂-terminal proANP (NT-proANP) (21), which is regarded as a more reliable marker of cardiac function than ANP. NH₂-terminal proBNP (NT-proBNP) is the circulating amino terminal fragment of the precursor of BNP (9). ANP is mainly synthesized in the atria of the normal adult heart, whereas BNP is principally released from ventricles (16, 36). It has been shown that plasma levels of ANP, NT-proANP, BNP, and NT-proBNP increase after myocardial infarction (16, 22, 23, 33, 34, 36, 42).

In addition to their systemic effects, ANP and BNP also directly modulate cardiac function. Both positive and negative inotropic effects of ANP and BNP have been reported (13, 20, 25). Studies have suggested that ANP inhibits cardiac hypertrophy as well (1, 7, 27). ANP has been shown to inhibit cardiac L-type Ca²⁺ channel activity (39) and its own secretion via type A natriuretic peptide receptors (NPR-A) (15). It has also been reported to inhibit DNA synthesis, proliferation, and collagen synthesis of cardiac fibroblasts in culture (3, 6, 32). In addition, mice lacking NPR-A or BNP develop cardiac fibrosis (19, 37). BNP has been shown to decrease collagen synthesis and activate cardiac matrix metalloproteinases as well (40). ANP and collagen I and III mRNA levels are increased in the adjacent noninfarcted myocardium as well as in the remote noninfarcted myocardium (44). Furthermore, radiation of the left ventricle induces ANP gene expression in the proximity of injured areas with degenerated myocytes and fibrosis (12). The main goal of the present study was to investigate the role of ANP and BNP in collagen formation and degradation and left ventricular remodeling in humans suffering acute myocardial infarction (AMI).

METHODS

Patients. We studied 93 consecutive patients admitted to Kuopio University Hospital between November 1998 and March 2000 for their first evolving AMI (30). Patients with typical chest pain and electrocardiogram showing ST segment elevation of ≥0.1 mV in at least two limb leads or ≥0.2 mV in at least two precordial leads were eligible for the study. The clinical characteristics of the patients are shown in Table 1. Patients were using the following cardiovascular drugs on admission to hospital: β-blockers (n = 19), calcium antagonists (n = 12), nitrates (n = 12), aspirin (n = 12), angiotensin-converting enzyme inhibitors (n = 11), diuretics (n = 4), digitals (n = 1), and hypolipidemic agents (n = 7). All patients gave their informed consent to the study. The investigation conformed with the principles outlined in the declaration of Helsinki and was approved by the local ethical committee.

Protocol. Patients were treated at the coronary care unit until stabilized. No definite time limits were set for the thrombolytic treatment. Either a total of 1.5 million IU streptokinase was infused over 60 min or reteplase regimen was used, in which a double bolus, at 1.5, 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, 96, and 120 h after AMI) from the laboratory.

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Submitted 27 May 2003; accepted in final form 5 November 2003

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type I procollagen (PINP), and carboxy terminal cross-linked telopeptide of type I collagen (ICTP) were assayed at entry, at 96 h, and at discharge from the hospital. Blood samples for natriuretic peptide al constitutes the data.

Table 1. Clinical characteristics

<table>
<thead>
<tr>
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<th>No. of Patients</th>
<th>%</th>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>66</td>
<td>77</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
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<tr>
<td>Hyperlipidemia</td>
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<td>59</td>
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<tr>
<td>Hypertension</td>
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</tr>
<tr>
<td>Diabetes</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>Smoking</td>
<td>53</td>
<td>62</td>
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<tr>
<td>ST-segment elevation</td>
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<td></td>
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<tr>
<td>Anterior</td>
<td>47</td>
<td>55</td>
</tr>
<tr>
<td>Inferoposterior</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Thrombolytic treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In hospital</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>Out of hospital</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>35</td>
<td>41</td>
</tr>
</tbody>
</table>

The majority of the patients (62%) developed Q-wave infarctions. TnT max was 4.5 μg/l (0.16–30.57) and the AUC of CK-MBm was 4.4 mg/l (0.1–21.9). There were no changes in PIIINP levels, whereas PINP levels decreased and ICTP levels increased after admission. The AUC of CK-MBm was 4.4 mg/l (0.1–21.9). There were no changes in PIIINP levels, whereas PINP levels decreased and ICTP levels increased after admission. The AUC of BNP increased from 3.8 pmol/l (0.1–1,828 pmol/l) to 124 pmol/l (41–1,473 pmol/l), and 9 pmol/l (3–68 pmol/l), respectively. There were no significant changes in

RESULTS

Clinical course of the patients. The time delay between the onset of symptoms and thrombolysis was 3.4 h (median 2.5 h, range 0.4–18.8 h). The average time between thrombolysis and admission to hospital was 1.9 h (median 1.8 h, range 0.3–4.5 h). Seven of the patients who received thrombolysis eventually presented with normal CK-MBm levels without permanent changes in electrocardiogram and were thus excluded. Thirty-seven patients used drug therapy for hypertension before AMI and were considered hypertensive. Fifty-six patients were considered reperfused on the basis of ST-segment returning to isoelectric or near isoelectric and chest pain relief shortly after thrombolysis. Sixteen patients experienced severe arrhythmic episodes, including nonsustained/sustained ventricular tachycardia and ventricular fibrillation. Twenty-nine patients suffered from some degree of clinical heart failure: 16 had mild pulmonary congestion, 11 had interstitial edema, and 2 had alveolar edema in chest X-rays. Two patients needed inotropic treatment at the coronary care unit. Fifteen patients had ejection fractions (EF) of <40% in two-dimensional echocardiography, and nine patients developed postthrombotic hemorrhage.

Infarct size and collagen markers. The majority of the patients (62%) developed Q-wave infarctions. TnT max was 4.5 μg/l (0.16–30.57) and the AUC of CK-MBm was 4.4 mg/l (0.1–21.9). There were no changes in PIIINP levels, whereas PINP levels decreased and ICTP levels increased after admission. The AUC of BNP increased from 3.8 pmol/l (0.1–1,828 pmol/l) to 124 pmol/l (41–1,473 pmol/l), and 9 pmol/l (3–68 pmol/l), respectively. There were no significant changes in
NT-proANP and NT-proBNP levels (Fig. 2). BNP levels increased after admission and remained elevated throughout the observation period \((P < 0.001, \text{Friedman test})\). NT-proANP levels, NT-proBNP levels, and BNP levels correlated with the AUC of CK-MBm and TnTmax at day 4 (Table 2).

On day 4, scar formation evaluated as serum PIIINP concentration correlated with NT-proANP \((r = 0.45, P < 0.001)\), NT-proBNP \((r = 0.50, P < 0.001)\) and with BNP \((r = 0.55, P < 0.001)\) (Table 2, Fig. 3). In addition, ICTP levels correlated with NT-proBNP \((r = 0.26, P < 0.05)\) and BNP \((r = 0.28, P < 0.05)\) on day 4. PINP levels did not correlate with NT-proANP, NT-proBNP, or BNP.

**Natriuretic peptides and left ventricular function.** Patients with left ventricular dysfunction \((\text{EF} < 40\%, n = 15)\) had similar NT-proANP levels as patients with well-preserved left ventricular function \((\text{EF} = 55 \pm 10\%, n = 57)\) at discharge \([534 (240–1,315) \text{ vs. } 397 (113–1,490) \text{ pmol/L}]\). However, BNP levels at discharge were higher in patients with left ventricular dysfunction than in patients with well-preserved left ventricular function \([56 (25–210) \text{ vs. } 18 (3–532) \text{ pmol/L}; P < 0.01]\). There were no significant differences in NT-proBNP levels between patients with well-preserved left ventricular function and patients with left ventricular dysfunction \([240 (42–3,125) \text{ vs. } 338 (202–1,941) \text{ pmol/L}]\).

NT-proANP correlated with the measured left ventricular internal end-systolic dimension \((r = 0.31, P < 0.05)\) and left ventricular EF \((r = -0.36, P < 0.01)\) (Table 3). BNP correlated with fractional shortening \((r = -0.36, P < 0.01)\), EF \((r = -0.41, P < 0.01)\), and left ventricular end-systolic volume \((r = 0.32, P < 0.05)\). NT-proBNP correlated with fractional shortening \((r = -0.32, P < 0.05)\), EF \((r = -0.34, P < 0.05)\), and left ventricular internal end-systolic dimension \((r = 0.33, P < 0.05)\).

**DISCUSSION**

In our study, NT-proANP, BNP, and NT-proBNP levels were elevated on admission and BNP levels increased further thereafter. These changes are in accordance with previous reports \((16, 22, 23, 33, 34, 36, 42)\). Plasma BNP and NT-proBNP levels reflected the degree of left ventricular dysfunction after AMI, which has been shown previously \((23, 33, 34)\). The main new finding in our study was that plasma NT-proANP, BNP, and NT-proBNP correlated with PIIINP levels, suggesting that ANP and BNP are involved in the regulation of collagen formation and thus left ventricular remodeling after AMI.

In humans, thrombolysis causes a nonorgan-specific transient release of PIIINP during the first hours after treatment, probably mediated by plasmin activation \((8, 28, 29)\). In the present study, blood samples were first taken at hospital, and 42% of the patients received thrombolytic treatment outside

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**Table 2.** Correlation coefficients of the AUC of CK-MBm, TnTmax, PIIINP, PINP, ICTP, and natriuretic peptides

<table>
<thead>
<tr>
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<th>On Day 4</th>
<th>At Discharge</th>
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<tr>
<td></td>
<td>NT-proANP</td>
<td>BNP</td>
</tr>
<tr>
<td>AUC of CK-MBm</td>
<td>0.30†</td>
<td>0.34†</td>
</tr>
<tr>
<td>TnTmax</td>
<td>0.36†</td>
<td>0.30†</td>
</tr>
<tr>
<td>PIIINP on day 4</td>
<td>0.45‡</td>
<td>0.55‡</td>
</tr>
<tr>
<td>at discharge</td>
<td>0.41†</td>
<td>0.31*</td>
</tr>
<tr>
<td>PINP on day 4</td>
<td>0.19±</td>
<td>-0.06</td>
</tr>
<tr>
<td>at discharge</td>
<td>0.17</td>
<td>-0.05</td>
</tr>
<tr>
<td>ICTP on day 4</td>
<td>0.3‡</td>
<td>0.26*</td>
</tr>
<tr>
<td>at discharge</td>
<td>0.16</td>
<td>0.20*</td>
</tr>
</tbody>
</table>

Area under curve (AUC) of creatine kinase MB mass (CK-MBm) and peak level of troponin T (TnTmax). Serum amino terminal propeptide of type III procollagen (PIIINP), intact amino terminal propeptide of type I procollagen (PINP), and carboxy terminal cross-linked telopeptide of type I collagen (ICTP) measured on day 4 and at discharge. NT-proANP, NH2-terminal pro-atrial natriuretic peptide; BNP, B-type natriuretic peptide; NT-proBNP, NH2-terminal proBNP. *P < 0.05; †P < 0.01; ‡P < 0.001, Spearman’s rank correlation test.
hospital. Therefore, contrary to previous studies (29, 41), we could not detect the acute changes in serum PIIINP levels. There is a secondary increase of PIIINP after 3 days, which is considered to reflect increased synthesis of type III collagen and myocardial healing (8, 28, 29, 41). We have previously shown that the PIIINP levels measured at days 4–10 correlate well with enzymatic infarct size and left ventricular function after AMI (41). In this study, a positive correlation was observed between the levels of PIIINP and the AUC of CK-MBm and TnTmax, confirming the earlier findings that the size of the infarct scar can be estimated from PIIINP concentration (28, 29, 41).

Myocyte stretch is the predominant stimulus controlling the release of ANP and BNP from the heart and produces an increase in ANP and BNP secretion within minutes (11, 14). Coronary artery ligation in sheep produces rapid increases of ANP, NT-proBNP, and BNP levels (31). Therefore, in the

<table>
<thead>
<tr>
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<th>NT-proANP</th>
<th>BNP</th>
<th>NT-proBNP</th>
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<tbody>
<tr>
<td>LVId, mm</td>
<td>55±8</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>LVIds, mm</td>
<td>40±9</td>
<td>0.31*</td>
<td>0.26</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>15±4</td>
<td>-0.05</td>
<td>-0.09</td>
</tr>
<tr>
<td>EDV, ml</td>
<td>131±50</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>ESV, ml</td>
<td>71±36</td>
<td>0.28</td>
<td>0.32*</td>
</tr>
<tr>
<td>FS, %</td>
<td>27±8</td>
<td>-0.18</td>
<td>-0.36†</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>51±13</td>
<td>-0.36†</td>
<td>-0.41†</td>
</tr>
</tbody>
</table>

Values are mean ± SD. LVId, left ventricular internal dimension end-diastole; LVIds, left ventricular internal dimension end-systole; IVS, interventricular septum end-systole; EDV, left ventricular end-diastolic volume; ESV, left ventricular end-systolic volume; FS, fractional shortening; LVEF, left ventricular ejection fraction (M-mode) 7.5 ± 1.4 days after admission. *P < 0.05; †P < 0.01, Spearman’s rank correlation test.

Fig. 3. Linear correlations between NT-proANP, BNP, NT-proBNP, PHINP, and ICTP on day 4. A: PIIINP vs. NT-proANP (r = 0.48, P < 0.001; left); ICTP vs. NT-proANP (r = 0.13, P = not significant; right). B: PIIINP vs. BNP (r = 0.52, P < 0.001; left); ICTP vs. BNP (r = 0.31, P < 0.01; right). C: PIIINP vs. NT-proBNP (r = 0.52, P < 0.001; left); ICTP vs. NT-proBNP (r = 0.39, P < 0.01; right).
present study, plasma levels on NT-proANP, NT-proBNP, and BNP were elevated already on admission to hospital (5). Unlike NT-proANP and NT-proBNP, BNP is a biologically active hormone. NT-proBNP and BNP are secreted in equimolar amounts (9), but only BNP binds to specific natriuretic peptide receptors and is removed from circulation. Furthermore, NT-proBNP has a slower clearance than the biologically active BNP (5). In our study, levels of BNP increased further after admission, whereas NT-proBNP levels remained elevated but did not change after admission. This is in accordance with the experimental data obtained in the sheep model (31). The differences in receptor binding and clearance probably explain the observed differences between NT-proBNP and BNP levels.

The distribution of NPR-A and NPR-B is relatively homogenous in the heart, whereas mRNA of the NPR-C is concentrated principally in the atria (26). Ventricular myocytes predominantly express the NPR-A, whereas in cardiac fibroblasts all three natriuretic peptide receptors have been identified (18). This suggests that ANP and BNP have autocrine or paracrine functions in the heart. Interestingly, mice lacking NPR-A develop cardiac fibrosis (19). BNP may be responsible for the antifibrotic activity, because there is cardiac fibrosis in mice lacking BNP (37). Although mice lacking ANP have hypertrophy without fibrosis (10), there is considerable in vitro data suggesting that ANP regulates the proliferation of fibroblasts. ANP has been shown to inhibit proliferation and synthesis of DNA and collagen in cultured cardiac fibroblasts (3, 6, 32). Furthermore, ANP is synthesized by fibroblasts infiltrating the infarct (2). Interestingly, irradiation of the left ventricle induces ANP gene expression in the proximity of injured areas with degenerated myocytes and fibrosis (12). In the present study, NT-proANP, BNP, and NT-proBNP correlated with PiIIINP, suggesting that ANP and BNP modulate collagen scar formation after AMI.

PINP is a marker of the biosynthesis of type I collagen, which is one of the most abundant components of extracellular matrix. In our study, levels of PINP decreased after admission. Most of the intact PINP in serum originates from bone matrix turnover, and decreased concentrations are likely to be due to the increased blood cortisol levels that occur after AMI (4, 28). ICTP cross-links with the helical domain of type I collagen and is removed from cross-links when type I collagen is degraded (35). ICTP levels increased after AMI in the present study, which has been shown previously as well (24). BNP has been shown to activate cardiac matrix metalloproteinases, which degrade matrix proteins (40). Interestingly, BNP and NT-proBNP correlated with ICTP, supporting the in vitro finding that BNP may regulate the degradation of collagen in heart (40).

Several studies have shown that ANP and BNP inhibit collagen synthesis and fibrosis in animal models and cell culture (2, 3, 6, 10, 32, 37, 40). To our knowledge, this is the first clinical study showing the correlation between natriuretic peptides and collagen synthesis after AMI in humans. Our results suggest that ANP and BNP may regulate collagen scar formation and left ventricular remodeling after AMI.

ACKNOWLEDGMENTS

The authors acknowledge Päivi Annala for performing the collagen assays and Tuula Lumijärv for performing the natriuretic peptide assays.

GRANTS

This work was supported by grants from Aarne Koskela Foundation, Maud Kuistila Foundation, and Academy of Finland.

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