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

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Maggi, Jarkko, Mikko Puhakka, Seppo Hietakorpi, Kari Punnonen, 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 NH2-terminal proANP (r = 0.45, P < 0.001), BNP (r = 0.55, P < 0.001) and NH2-terminal 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 NH2-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.

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-convertase enzyme inhibitors (n = 11), diuretics (n = 4), digitalis (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 intravenous bolus of 10 mg was given. All other medications were allowed.

Serum samples for mass of the myocardial isof orm of creatine kinase (CK-MBm) assays were taken before treatment (when feasible), at 1.5, 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, 96, and 120 h after thrombolysis, and at discharge (day 9 or 10 after AMI) from the hospital. Troponin T (TnT) was assayed before treatment (when feasible), at 6, 48, 72, 96, and 120 h after thrombolysis, and at discharge from the hospital. ANP and BNP were assayed at entry, at 96 h, and at discharge from the hospital. Amino terminal propeptide of type III procollagen (PIIIIP), intact amino terminal propeptide of

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

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coefficients of variation in each assay were 0.5, 30, and 40 pmol/l plasma, respectively. The within- and between-assay coefficients of variation (CV) were 10 and 15%, respectively. None of the assays have any cross-reactivity (0.1%).

Electrocardiogram and chest radiographs were taken when clinically relevant. Echocardiography was performed at an average of 7.5 ± 1.4 days after admission to hospital.

Laboratory analysis. CK-MBm and TnT measurements were performed at Kuopio University Hospital laboratory with the use of standard methods. The area under the curve (AUC) of CK-MBm and peak TnT level (TnTmax) were used to estimate the size of the infarcts. Both AUC of CK-MBm and TnTmax have been shown to be reliable tools for serological estimation of myocardial infarct size independent of early coronary reperfusion (17). The concentrations of serum PIIINP, PINP, and ICTP were analyzed with commercially available radioimmunoassays (Orion Diagnostica, Espoo, Finland). BNP was extracted from plasma by using SepPak C18 cartridges. NT-proANP and NT-proBNP were assayed directly from 25 μl of unextracted plasma. The assays utilized protocols described previously (43). The sensitivities of the BNP, NT-proANP, and NT-proBNP assays were 0.5, 30, and 40 pmol/l plasma, respectively. The within- and between-assay coefficients of variation in each assay were <10 and <15%, respectively. None of the assays have any cross-reactivity (<0.1%) against the other peptides, nor against ANP or C-type natriuretic peptide.

Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Male</td>
<td>66</td>
<td>77</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>23</td>
</tr>
</tbody>
</table>

Risk factors

- Hyperlipidemia: 51 (59)
- Hypertension: 37 (43)
- Diabetes: 26 (30)
- Smoking: 53 (62)

ST-segment elevation

- Anterior: 47 (55)
- Inferoposterior: 39 (45)

Thrombolytic treatment

- In hospital: 50 (58)
- Out of hospital: 36 (42)

Thrombolysis

- Streptokinase: 51 (59)
- Reteplase: 35 (41)

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 and collagen marker assays were immediately spun, and the plasma and serum were stored at −70°C until analysis.

Infarct size and collagen markers.

The majority of the patients (62%) developed Q-wave infarctions. TnTmax 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 (Fig. 1) (P < 0.001, Friedman test). On day 4, PIIINP correlated with AUC of CK-MBm (r = 0.29, P < 0.05) and with TnTmax (r = 0.33, P < 0.01). PINP or ICTP levels on day 4 did not correlate with AUC of CK-MBm or TnTmax.

Relationships of natriuretic peptides with infarct size and collagen markers.

The median plasma levels of NT-proANP, NT-proBNP, and BNP on admission to hospital were 345 pmol/l (78–1,473 pmol/l), 124 pmol/l (41–1,473 pmol/l), and 9 pmol/l (3–68 pmol/l), respectively. There were no significant changes in

Statistical analysis. Nonparametric tests were used, because levels of NT-proANP, BNP, NT-proBNP, CK-MBm, TnT, PIIINP, PINP, and ICTP were not normally distributed. Values are given as medians (range) or means ± SD as appropriate. Friedman’s test, Spearman’s rank correlation test, and the Mann-Whitney test were used to analyze the data.

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 given 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 isolectric or near isolectric 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 postthrombolytic hemorrhage.

A

Fig. 1. Serum levels (in μg/l) of amino terminal propeptide of type III procollagen (PIIINP; A), amino terminal propeptide of type I procollagen (PINP; C), and carboxy terminal cross-linked telopeptide of type I collagen (ICTP; B). Levels of PIIINP, PINP, and ICTP are shown in boxes (25–75% percentiles), with the whiskers showing range and the horizontal lines showing median.
NT-proANP and NT-proBNP levels (Fig. 2). BNP levels increased after admission and remained elevated throughout the observation period (P < 0.001, Friedman test). NT-proANP levels, NT-proBNP levels, and BNP levels correlated with the AUC of CK-MB and TnT 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 (EF < 40%, n = 15) had similar NT-proANP levels as patients with well-preserved left ventricular function (EF = 55 ± 10%, n = 57) at discharge [534 (240–1,315) vs. 397 (113–1,490) 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) vs. 18 (3–532) 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) vs. 338 (202–1,941) 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.

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

<table>
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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></td>
<td></td>
<td></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>PINP on day 4</td>
<td>0.41†</td>
<td>0.31*</td>
</tr>
<tr>
<td>ICTP on day 4</td>
<td>0.19</td>
<td>−0.06</td>
</tr>
<tr>
<td>PINP on discharge</td>
<td>0.17</td>
<td>0.28*</td>
</tr>
<tr>
<td>ICTP on discharge</td>
<td>0.16</td>
<td>0.29*</td>
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</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

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Table 3. Echocardiographic parameters and correlation coefficients of echocardiographic parameters and natriuretic peptides at discharge

<table>
<thead>
<tr>
<th></th>
<th>NT-proANP</th>
<th>BNP</th>
<th>NT-proBNP</th>
</tr>
</thead>
<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>
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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-diastole; ESV, left ventricular end-systole; 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.
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 PIIINP, 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 and is removed from cross-links when type I collagen is altered effect in experimental hypertension. Atrial natriuretic factor— a circulating hormone stimulated by volume loading. The amino-terminal portion of pro-brain natriuretic peptide (Pro-BNP) circulates in human plasma. Biochem Biophys Res Commun 214: 1175–1183, 1995.


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REFERENCES


Natriuretic Peptides and Collagen Metabolic Markers


