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

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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

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Magga, 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 the 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.

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 (10 million IU) was given over 60 min, followed by a continuous infusion of 1.0 million IU/h. Thrombolytic therapy was continued for up to 6 h, and a total of 1.5 million IU streptokinase or 10 million IU reteplase was given in the majority of patients.

One and four days after admission, blood samples were taken before the start of thrombolytic therapy and immediately after initiation of the infusion. Blood samples were collected in heparinized tubes for plasma ANP and BNP measurements and in fluoride-EDTA tubes for measurement of cardiac enzymes.

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Table 1. Clinical characteristics

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<td>Thrombolytic treatment</td>
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<tr>
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<tr>
<td>Repletase</td>
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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 and collagen marker assays were immediately spun, and the plasma and serum were stored at −70°C until analysis.

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 (TnT_max) were used to estimate the size of the infarcts. Both AUC of CK-MBm and TnT_max 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.

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 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 postthrombolytic 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/h/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 TnT_max (r = 0.33, P < 0.01). PINP or ICTP levels on day 4 did not correlate with AUC of CK-MBm or TnT_max.

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 (78–1,828 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

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-MBm and TnT_max 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...
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, con-
fiming 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

![Fig. 3. Linear correlations between NT-proANP, BNP, NT-
proBNP, PIIINP, 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.]

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<tr>
<th>Echocardiographic parameters and correlation coefficients of echocardiographic parameters and natriuretic peptides at discharge</th>
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<tr>
<td>LVIDd, mm</td>
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<td>LVIDs, mm</td>
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<td>LVEF, %</td>
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Values are mean \pm SD. LVIDd, left ventricular internal dimension end-
diastole; LVIDs, left ventricular internal dimension end-systole; IVS, inter-
ventricular 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 \pm 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 preenous in the heart, whereas mRNA of the NPR-C is concen-

the observed differences between NT-proBNP and BNP levels.

The amino-terminal portion of pro-brain natriuretic peptide (Pro-BNP) circulates in human plasma. The amino-terminal portion of pro-brain natriuretic peptide (Pro-BNP) 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.

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GRANTS

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