Myocardial Protective Effect of Human Atrial Natriuretic Peptide in Cardiac Surgery

– “hANP Shot” in Clinical Safety Trial –

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Background: We studied low-dose human atrial natriuretic peptide (hANP) infusion therapy during cardiac surgery and reported the cardiac and renal protective effects. The efficacy of a bolus injection of hANP (the “hANP shot”) simultaneously with induction of cardioplegia has been proven in animal experiments. In the present study the clinical effects of this “hANP shot” were examined.

Methods and Results: The subjects were 67 patients undergoing Coronary artery bypass grafting. At the time of inducing cardioplegia, 1 group received a simultaneous bolus injection of 100 μg of hANP (hANP group) and the other group received an injection of physiological saline (placebo group). The primary endpoints were (1) operative mortality and complications, and (2) the creatine kinase isoenzyme MB (CPK-MB), troponin-I, and human heart fatty acid binding protein (H-FABP) levels. The secondary endpoints were (1) the incidence of arrhythmia, and levels of (2) atrial and B-type natriuretic peptides, and cyclic guanosine monophosphate (cGMP), and (3) renin, angiotensin II, and aldosterone. Postoperative CPK-MB, troponin-I, and H-FABP levels were significantly lower in the hANP group than in the placebo group. Postoperative arrhythmia was significantly less frequent in the hANP group than in the placebo group.

Conclusions: It is possible to achieve cardioprotective effects based on the safety of the “hANP shot”, as well as from biomarkers of ischemia and results related to arrhythmia. The “hANP shot” should also be evaluated as a safer and new cardioprotective method for cardiac surgery. (Circ J 2011; 75: 2144–2150)

Key Words: Atrial natriuretic peptide; Coronary artery bypass grafts surgery; Natriuretic peptides

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“hANP Shot” in Clinical Safety Trial

Methods

Study Protocol

The hANP shot clinical trial was a randomized double-blind placebo-controlled study of patients underwent isolated coronary artery bypass grafting (CABG) with CPB. The patients with cardiogenic shock, acute myocardial infarction, arrhythmia or a history of arrhythmia, hemodialysis, clinical hypothyroidism or hyperthyroidism, or having off-pump CABG were excluded. Patients requiring 2 injections of cardioplegia because of the long period of aortic clamping were excluded. Patients were randomized into 2 groups by the lottery method. After aortic clamping, when 4°C cold cardioplegia (St. Thomas’s Hospital I solution) was injected, hANP (carperitide) dissolved in 5 ml of physiological saline (Daiichi-Sankyo Pharmaceutical Co Ltd, and Asbio Pharmaceutical Inc Co Ltd, Kobe, Japan) was administered as a 100-μg bolus injection (hANP group) or only 5 ml of physiological saline was administered (placebo group) via the lateral branch of the cardioplegia injection route under blinded conditions. When we performed administration of cardioplegia mixed with hANP in previous animal research, hANP was adsorbed in the cardioplegia administration circuit and it was confirmed experimentally that hANP was not injected into the heart. Therefore, we performed a bolus injection and compared experimental doses of 25 μg and 100 μg. Because we found that the 100-μg dose showed stronger cardioprotective effects and did not have adverse effects on the hemodynamics, a dose of 100 μg was used in this study. It was conducted after obtaining approval from the Ethics Committee of Nihon University School of Medicine Itabashi Hospital. Informed consent was provided by each patient after they received an explanation about the study. This study was registered with the University Hospital Medical Information Network (UMIN) (study ID: UMIN000001469).

CABG was performed with CPB (Jostra HL-20, Jostra Inc, Hirrlingen, Germany) and nonpulsatile perfusion at a tepid temperature (rectal temperature: 34°C). St. Thomas’s Hospital I solution (4°C) was used as cardioplegia at the initial dose of 30 ml/kg. Thereafter, antegrade administration was undertaken at 10 ml/kg at 60-min intervals [7]. When cardioplegia was induced, hANP dissolved in physiological saline or only physiological saline was injected by a bolus injection at the same time using a 5 ml syringe via the lateral branch of the cardioplegia injection route. Because our previous research showed that continuous administration of low-dose hANP was effective in terms of cardiac and renal protective effects in cardiac surgery using CPB [11-14], all patients received continuous infusion of hANP (0.02 μg·kg⁻¹·min⁻¹) from the beginning of CPB via the central venous pressure catheter. In all patients, the left internal thoracic artery was anastomosed to the left
anterior descending coronary artery, and radial artery and/or saphenous vein grafts were used for other vessels.

Endpoints

The primary endpoints were (1) postoperative creatine kinase isoenzyme MB (CPK-MB), troponin-I and human heart fatty acid binding protein (H-FABP) levels, and (2) operative mortality and complications. The CPK-MB was measured on return to the intensive care unit (ICU), 3 h after returning, and on postoperative Days 1 and 3. The troponin-I and H-FABP levels were measured on return to the ICU, and on postoperative Days 1 and 3. Postoperative complications were categorized as central nervous system disorders, cardiac events [low output syndrome, heart failure, perioperative myocardial infarction, and drug-induced arrhythmia], respiratory failure, acute renal failure (requiring dialysis), gastrointestinal disorders (gastrointestinal bleeding, ileus, or a requirement for high-calorie alimentation such as in patients with liver dysfunction), infections (septicemia, pneumonia, mediastinitis, etc), and other disorders requiring long-term ICU management. The definition of heart failure in this study was pulmonary congestion observed on chest X-rays and inability to maintain hemodynamics (systolic blood pressure ≤ 90 mmHg) even with administration of 5 μg·kg⁻¹·min⁻¹ or more of dopamine or dobutamine. The pulse rate was monitored for 1 week after surgery by continuous ECG monitoring. Occurrence of arrhythmia was defined as persistence of arrhythmia for ≥5 min or a requirement for treatment because of hemodynamic changes.

The secondary endpoints were (1) occurrence/non-occurrence of arrhythmia during the initial 1-week period after surgery, (2) atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and cGMP levels, and (3) plasma renin activity, and angiotensin-II, aldosterone and serum creatinine (sCr) levels. The ANP, BNP, cGMP, renin activity, angiotensin-II, aldosterone and sCr were measured before surgery, on return to ICU, and on postoperative Days 1, 3 and Week 1.

Statistical Analysis

Data are expressed as the mean±standard deviation of the mean. For parametric and non-parametric data, statistically significant differences were determined by Student’s t-test and Fisher’s exact test, respectively. A P-value less than 0.05 was considered to indicate statistical significance. Other data were analyzed by using repeated measures ANOVA. All analyses were conducted with SPSS software (SPSS Inc, IL, USA).

Results

Patient Enrolment

We enrolled 70 patients excluding 14 patients who were excluded from or refused enrollment. In the hANP group, 2 patients who underwent other operative procedures simultaneously (mitral annuloplasty, ascending aorta replacement) and in the placebo group, 1 patient given 2 injections of cardioplegia, were excluded. Therefore, 67 subjects were examined, consisting of 33 in the hANP group and 34 in the placebo group (Figure 1).

Baseline Characteristics

Preoperative patient characteristics showed no significant differences between the 2 groups, including age, male/female ratio, body surface area, underlying diseases, emergency surgery, risk factors and preoperative medications (Table 1).
Table 2. Operative and Postoperative Data

<table>
<thead>
<tr>
<th></th>
<th>hANP group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>33</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>ACCT (min)</td>
<td>60.0±1.7</td>
<td>57.0±2.7</td>
<td>0.3514</td>
</tr>
<tr>
<td>CPBT (min)</td>
<td>104.9±3.6</td>
<td>103.6±3.9</td>
<td>0.8088</td>
</tr>
<tr>
<td>Bypass</td>
<td>3.2±0.1</td>
<td>3.2±0.1</td>
<td>0.8648</td>
</tr>
<tr>
<td>EC after declamping</td>
<td>1 (3.0%)</td>
<td>6 (17.6%)</td>
<td>0.1054</td>
</tr>
<tr>
<td>Dopamine use</td>
<td>2 (6.1%)</td>
<td>4 (11.8%)</td>
<td>0.6728</td>
</tr>
<tr>
<td>Dobutamine use</td>
<td>1 (3.0%)</td>
<td>2 (5.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Norepinephrine use</td>
<td>0 (0%)</td>
<td>1 (2.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>0 (0%)</td>
<td>1 (2.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>180 days</td>
<td>0 (0%)</td>
<td>1 (2.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Complications</td>
<td>0 (0%)</td>
<td>2 (5.9%)</td>
<td>0.4925</td>
</tr>
<tr>
<td>Postoperative arrhythmia</td>
<td>3 (9.1%)</td>
<td>11 (32.4%)</td>
<td>0.0333</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2 (6.1%)</td>
<td>6 (17.6%)</td>
<td>0.2585</td>
</tr>
<tr>
<td>PVC</td>
<td>1 (3.0%)</td>
<td>5 (14.7%)</td>
<td>0.2974</td>
</tr>
</tbody>
</table>

hANP, human atrial natriuretic peptide; ACCT, aortic cross-clamp time; CPBT, cardiopulmonary bypass time; EC, electrical cardioversion; PVC, premature ventricular contraction.

![Figure 2](image-url). Changes in atrial and B-type natriuretic peptides (ANP, BNP) and cyclic-guanosine monophosphate (GMP). hANP, human ANP; ICU, intensive care unit.
Operative and Postoperative Data

Table 2 shows the number of bypasses, aortic cross-clamping time, and CPB time. No significant differences were observed between the hANP and placebo groups. Isosorbide dinitrate was used in all patients postoperatively. Use of dopamine, dobutamine, and norepinephrine showed no significant differences between the 2 groups. There were no major adverse events, such as shock or acute renal failure, that could be considered to be caused by the “hANP shot”.

Primary Endpoints

Postoperative Outcome The 30- and 180-day mortality rate was 0% in the hANP group and 2.9% (n=1) in the placebo group, with the cause of death being cerebral infarction in the 1 patient. No significant difference was found between the 2 groups with respect to 30- and 180-day mortality (P=1.00). Perioperative complications occurred in 2 patients (5.9%) from the placebo group: heart failure in 1 and cerebral infarction in another patient. The incidence of complications was not significant between the 2 groups (P=0.4925).

Postoperative CPK-MB, Troponin-I and H-FABP Levels (Figure 2) CK-MB was 26.4±1.5 U/L in the hANP group and 34.6±3.1 U/L in the placebo group at 3 h after surgery, and 17.2±1.2 ng/ml and 23.7±2.6 ng/ml, respectively, on postoperative Day 1. The hANP group showed significantly lower values at both 3 h after surgery (P=0.0218), and Day 1 (P=0.0259). On postoperative Day 1, troponin-I was 2.7±0.4 ng/ml in the hANP group and 5.0±0.7 ng/ml in the placebo group, with the hANP group having a significantly lower level (P=0.0058). H-FABP was 39.5±7.6 ng/ml in the hANP group and 57.6±6.0 ng/ml in the placebo group on return to ICU, with the hANP group having a significantly lower level (P=0.0085).

Secondary End Points

Postoperative Arrhythmia Patients who underwent electric cardioversion after declamping were significantly fewer in the hANP group and the self-rate occurred spontaneously after declamping in many patients (P=0.0544). Postoperative arrhythmia occurred in 3 patients (5.9%) from the hANP group and 11 patients (32.4%) from the placebo group. The incidence of postoperative arrhythmia was significantly lower in the hANP group than in the placebo group (P=0.0333). One patient from the hANP group (3.0%) had ventricular arrhythmias requiring postoperative treatment vs. 5 patients from the placebo group (14.7%). Atrial fibrillation occurred in 2 patients from the hANP group (6.1%) and 6 patients from the placebo group (17.6%).

ANP, BNP and cGMP Levels (Figure 3) On return to ICU, the ANP level was 524.0±37.5 pg/ml in the hANP group and 390.0±25.5 pg/ml in the placebo group, with the hANP group having a significantly lower ANP level (P=0.0042). On return to ICU, the cGMP level was 35.2±3.7 pmol/ml in the hANP group and 19.0±2.9 pmol/ml in the placebo group, with the hANP group having a significantly higher cGMP level (P=0.0009). For BNP, there were no significant differences.
between the groups.

**Plasma Renin Activity, Angiotensin-II, Aldosterone and sCr Levels** (Table 3) There were no pre- or postoperative differences in RAAS and sCr between the 2 groups.

### Table 3. Renin-Angiotensin-Aldosterone System and Renal Function After Operation

<table>
<thead>
<tr>
<th></th>
<th>Preoperative</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Week 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renin activity (ng·ml⁻¹·h⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hANP</td>
<td>6.88±1.66</td>
<td>17.60±3.34</td>
<td>10.40±2.34</td>
<td>10.29±2.45</td>
<td>11.87±1.85</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.58±1.49</td>
<td>15.41±3.56</td>
<td>9.65±2.71</td>
<td>8.37±1.45</td>
<td>13.92±2.35</td>
</tr>
<tr>
<td><strong>Angiotensin-II (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hANP</td>
<td>17.30±3.46</td>
<td>39.92±6.54</td>
<td>28.71±4.19</td>
<td>23.42±4.21</td>
<td>26.39±4.60</td>
</tr>
<tr>
<td>Placebo</td>
<td>13.73±2.10</td>
<td>34.55±4.71</td>
<td>26.84±5.31</td>
<td>25.06±3.49</td>
<td>23.12±3.37</td>
</tr>
<tr>
<td><strong>Aldosterone (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hANP</td>
<td>60.53±4.75</td>
<td>89.1±9.79</td>
<td>69.32±7.56</td>
<td>65.00±6.79</td>
<td>68.35±6.16</td>
</tr>
<tr>
<td>Placebo</td>
<td>66.03±9.69</td>
<td>99.12±11.68</td>
<td>75.18±13.44</td>
<td>73.36±9.94</td>
<td>72.67±10.12</td>
</tr>
<tr>
<td><strong>Serum creatinine (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hANP</td>
<td>0.98±0.05</td>
<td>0.95±0.42</td>
<td>1.12±0.09</td>
<td>0.93±0.06</td>
<td>0.96±0.05</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.98±0.05</td>
<td>0.98±0.05</td>
<td>1.07±0.06</td>
<td>0.94±0.06</td>
<td>0.98±0.07</td>
</tr>
</tbody>
</table>

hANP, human atrial natriuretic peptide.

**Discussion**

In the present pilot study, the safety of the “hANP shot” was confirmed based on operative mortality and effects on the onset of complications. The preventive effects on ischemic reperfusion disorders and the antiarrhythmic effect of the “hANP shot” were also proven from the results for markers of myocardial injury and onset of arrhythmia. Damage to the myocardium for which the biomarkers of myocardial injury are not very high is considered to have little clinical effect, but recently there have been reports that the CK-MB and troponin I levels within 24h postoperatively are involved in postoperative mid-term and long-term mortality. Therefore, it was considered necessary to evaluate not only the short-term but also the long-term conditions.

Recently, continuous intravenous administration of hANP or BNP (nesiritide) have been performed in cardiac surgery worldwide, and cardiac and reno-protective effects have been reported.11-14,19,22 hANP is only used in Japan at present, because BNP has not been approved in Japan, but is used in Western countries. The NAPA trial of the use of BNP during cardiac surgery was performed for patients with left ventricular dysfunction in the United States. In the BNP group, the peak sCr was lower and the glomerular filtration rate was higher, and the survival rate at 180 days postoperatively was significantly higher in the BNP group than in the placebo group.21 A trial of BNP in patients with preoperative renal dysfunction was reported by the Mayo Clinic, showing better postoperative results in the BNP group, in which the plasma cystatin and aldosterone levels and estimated creatinine clearance were better than in the placebo group.22 Continuous intravenous injection of hANP or BNP during cardiac surgery was found to be effective from both the cardiac function and renal function aspects. However, this is the first clinical trial to perform a direct injection based on the concept of cardioplegia during cardiac surgery. Experimental reports are limited to the 2 from our institute15,16 and the report by Lazar et al.23 We evaluated the “hANP shot” by experimental study, in which blood and myocardial cGMP and myocardial ATP levels were significantly higher in the hANP group than in the control group. Myocardial calcium concentrations were significantly lower in the hANP group than in the control group. By electron microscopy, ischemic reperfusion injury was rarely observed in the hANP group. The “hANP shot” improves ischemic reperfusion injury and we have suggested that hANP exerts direct myocardial protection against myocardial injury associated with cardiac surgery.15,18 In research on BNP, Lazar et al reported that the infarct sized decreased significantly in the BNP group when compared with the untreated group, and endothelial function was protected in the BNP group.24 Comparative research on hANP and BNP has been reported only by Kimura et al25 and in a report on the administration of BNP to treat heart failure, which stated that renal function worsened because of hypotension.25 hANP has a shorter half-life than BNP24 and it will be necessary to perform a comparative study of the 2 agents in the future. From the safety standpoint, we think that hANP is better.

In the early postoperative period, cGMP, the hANP second messenger, was significantly higher in the hANP group than in the placebo group, indicating that hANP has cardioprotective effects via the cGMP route. cGMP activates protein kinase, inhibits the inflow of calcium into cells and has vasodilator and myocardial protective actions.26-28 In our animal experiments, we proved that hANP inhibits calcium overload in the myocardium via cGMP.15,16 Because only the efficacy of a “hANP shot” was examined in the present study, low-dose continuous administration of hANP during surgery should not be performed. The results of this research suggest that in the patients in whom the “shot” is not administered, not only cGMP, the hANP second messenger, but also ANP and other myocardial damage markers might show greater differences. However, we have evidence that when hANP is used during cardiac surgery, it is effective from the aspects of cardiac and renal protection, not only from early postoperatively but also in the long-term.14 In cardiac surgery using CPB, we plan to administer hANP continuously at low doses and study all patients under continuous treatment. However, during the period of aortic clamping, hANP administered continuously at low doses will circulate systemically outside the heart and lungs and there will be almost no circulation in the heart. It was verified from this study that intracoronary injection of hANP immediately before aortic clamping is effective.

The final goal of this study was to determine if hANP has adequate cardioprotective effects even when the aortic cross-clamping time is prolonged. The cardioprotective effects were clear from this pilot study but we hope to continue this research in the future to further clarify the efficacy and problems, and
finally to evaluate the efficacy of the “hANP shot” by prolonging the aortic clamping time.
In this study, the subjects were isolated CABG patients but this method might be more effective in patients with long aortic clamping times, with left ventricular dysfunction, with myocardial hypertrophy because of aortic valve stenosis, etc, as well as in acute myocardial infarction patients, and further study is warranted.

Study Limitations
In the present study, hANP low-dose continuous administration was performed simultaneously and to verify the efficacy of only the “hANP shot”, it is possible that not performing hANP low-dose continuous administration would be better for proving the “hANP shot” efficacy. If ANP and cGMP were examined in blood drawn from the coronary sinus, the values might show a greater difference. It was possible to show only that hANP reduced ischemic reperfusion in relation to the cause of onset of arrhythmia, but in the future, various markers, such as inflammatory markers, should be measured and a detailed study must be performed of the arrhythmia preventive effects of hANP.

Conclusions
The “hANP shot” based on a new concept of injection into the myocardium together with a cardioplegia was found to be clinically safe. From the ischemia biomarkers and effects on arrhythmia, it appears that the “hANP shot” has cardioprotective effects. In this study, an evaluation was performed on concomitant use of low-dose maintenance treatment based on extracorporeal circulation of hANP. In the future, we hope to perform a trial of the “hANP shot” only and to evaluate further the “hANP shot” as a new cardioprotective method.

Acknowledgments
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Disclosures
None of the authors have any conflicts of interest associated with this study.

References