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Original Article

Experimental investigation of "hANP shot" using human atrial natriuretic peptide for myocardial protection in cardiac surgery

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ABSTRACT

Background: We investigated myocardial protection by human atrial natriuretic peptide (hANP) during cardiac surgery without cardioplegia and determined whether suppression of myocardial ischemic reperfusion injury by hANP allows intraoperative aortic cross-clamp time to be prolonged.
Methods and results: Thirty-two pigs were placed on cardiopulmonary bypass. Experimental pigs were divided into 4 groups: 15 min clamping; hANP 15 min clamping; 30 min clamping; and hANP 30 min clamping. In both hANP groups, a 100 μg dose of hANP was administered after clamping. Left ventricular function, premature ventricular contractions (PVCs), histopathological studies, 8-isoprostane, myocardial Ca^{2+}, and ATP concentrations were determined. Comparison of the myocardial contractile force indicator E_{max}, in the 30 min groups, showed a significantly higher recovery rate in the hANP than in the control group. PVC numbers were significantly lower in the hANP than in the control groups for both arrest durations. On microscopic examination, hANP reduced ischemic reperfusion injury in the 30 min groups. The myocardial ATP level was significantly higher in the hANP 30 min than in the control 30 min group. Increases in 8-isoprostane and myocardial Ca^{2+} concentrations were significantly inhibited in both hANP groups.
Conclusions: This study demonstrated that hANP ameliorates ischemic reperfusion injury, improves postoperative myocardial contractility, and reduces reperfusion arrhythmias. We suggest that hANP allows aortic cross-clamping to be prolonged and thereby exerts a direct myocardial protective effect against cardiac arrest during cardiac surgery.

Introduction

Human atrial natriuretic peptide (hANP; carperitide, Suntory Inc., Osaka, Japan, and Daiichi Sankyo Co., Ltd., Tokyo, Japan) exerts various pharmacological actions including natriuretic and vasodilatory activities, as well as myocardial protection [1–3]. hANP has currently been used to treat patients with heart failure by natriuretic actions, vasodilatory actions, and inhibition of the renin-angiotensin system [3,4]. Several studies have demonstrated beneficial effects of hANP on experimentally induced ischemic reperfusion myocardial injury [5,6]. Kuga et al. reported that hANP administration, combined with percutaneous coronary intervention, suppressed reperfusion phenomena and preserved left ventricular function through improvement of regional wall motion [6]. An inhibitory effect on left ventricular remodeling, reduced left ventricular arrhythmias, and ejection fraction maintenance after ischemic reperfusion have also been demonstrated [5–7]. Kitakaze et al. reported a randomized trial (J-WIND) in which hANP was administered as an adjunct to reperfusion treatment for acute myocardial infarction [8]. They showed that adjunctive, acute-phase treatment with hANP after reperfusion therapy in patients with acute myocardial infarction reduced infarct size and increased the left ventricular ejection fraction during the chronic phase. hANP has also been demonstrated to suppress the renin-aldosterone system, decrease sympathetic nervous system activity, and dilate coronary arteries [3]. In addition, hANP has been proposed to inhibit endothelin-1, inflammatory cytokines, and tumor necrosis factor-α, as well as reducing oxygen radical cell damage and calcium overload after ischemic reperfusion [9,10].

We have suggested the efficacy of low dose continuous infusion of hANP during cardiopulmonary bypass (CPB) during cardiac surgery [11,12]. It has been recognized that hANP decreases development of arrhythmias during unstable conditions after cardiac surgery. An inhibitory effect on left ventricular remodeling and reduced ischemic reperfusion injury have been reported by administering an intravenous infusion of hANP during cardiac surgery

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[13,14]. Therefore, we anticipated that hANP would be efficacious in achieving intraoperative myocardial protection. Our previous study has already demonstrated that intracoronary administration of hANP 100 μg to pig hearts as an adjuvant to cardioplegia reduced ischemic reperfusion injury [15,16].

The effect on left ventricular function and arrhythmias after ischemic reperfusion injury were not thoroughly investigated in the previous study. Therefore, we evaluated the left ventricular function by E_{max} and Tau, and then we measured the number of premature ventricular contractions (PVCs) after reperfusion in the present study. Furthermore, the present study aimed to reveal whether hANP allows the aortic cross-clamp time to be prolonged. We investigated whether hANP alone inhibits myocardial ischemic reperfusion injury without cardioplegia during cardiac arrest.

Materials and methods

Experiment preparation

Thirty-two male pigs (41 ± 2.2 kg) were used for the experiments. An intramuscular injection of pentobarbital sodium (20 mg/kg) was administered as premedication and anesthesia was maintained with continuous intravenous infusion of ketamine hydrochloride (1 mg/kg/h). After tracheal intubation, controlled mechanical ventilation was established at 20–25 breaths/min with a tidal volume of 10–15 mL/kg by means of volume-controlled ventilation (Servo 900-E, Siemens-Elema Inc., Stockholm, Sweden). Limb lead electrocardiograms were employed for monitoring the conditions of the animals. A venous line was inserted into the right internal jugular vein for injection. The left femoral artery was cannulated to monitor continuous aortic pressure. A catheter (CX-654U, Cathex Co., Ltd., Tokyo, Japan) was placed in the coronary sinus via the left internal jugular vein for blood collection. The thorax was opened in the supine position via median sternotomy, after systemic heparinization (300 U/kg). A dual-stage venous cannula (TF-024-L, Research Medical Inc., Midvale, Utah, USA) was placed in the right atrium, and an aortic cannula (Medtronic Inc., Minneapolis, MN, USA) was inserted into the ascending aorta. A gyro pump (Kyocera Corp., Kyoto, Japan) and an oxygenator [HPO-20-HC, Senko IKEA Kogyo (Mara), Tokyo, Japan] were prepared for CPB. A vent tube was inserted through the left atrium into the left ventricle.

All animals received humane care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html).

Experimental groups

Cardiac surgery models

Thirty-two pigs were placed on CPB with mild hypothermia at 27 °C, and 5 min thereafter aortic cross-clamping was performed with electrically induced ventricular fibrillation. During aortic cross-clamping, the blood was re-warmed to 32 °C. This method approximates the intermittent aortic cross-clamping (IAC) which is one of the protective procedures applied during coronary artery bypass grafting (CABG) [17]. We use IAC for CABG in our institute. Experimental pigs were divided into 4 groups: 15 min clamping (control 15 min group, $n = 8$); hANP 15 min clamping (hANP15 min group, $n = 8$); 30 min clamping (control 30 min group, $n = 8$); and hANP 30 min clamping (hANP 30 min group, $n = 8$). A 100 μg (20 μg/mL 5 mL) dose of hANP was administered via the aorta near the aortic clamper using a 27 G needle immediately after clamping. Then, the aorta was flushed with 10 mL of normal saline in hANP groups. The control groups received 15 mL flushes with normal saline. At 20 min after declamping, the animals were weaned from CPB (Fig. 1). No inotropic drugs were administered during the study. Fresh tissue was taken from the inner layer of the left ventricular free wall after completion of the experimental protocol.

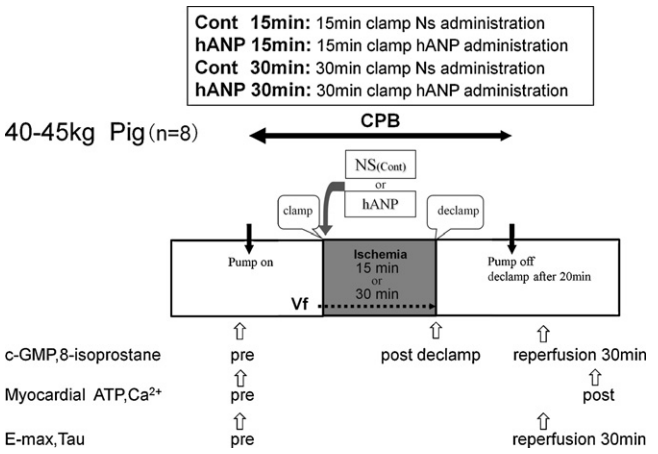

Fig. 1. Experimental method and groups. Thirty-two pigs were divided into 4 groups; 15 min clamping (control 15 min group $n = 8$), hANP 15 min clamping (hANP 15 min group $n = 8$), 30 min clamping (control 30 min group $n = 8$) and hANP 30 min clamping (hANP 30 min group $n = 8$). A 100 μg dose of hANP (20 μg/mL 5 mL) was administered via the aorta after clamping. Cont, control; Ns, normal saline; hANP, human atrial natriuretic peptide; CPB, cardiopulmonary bypass.

Biochemical parameters

Plasma cyclic guanosine monophosphate (c-GMP) and 8-isoprostane (prostaglandin F2α) were measured in samples collected from the coronary sinus at 3 time points: before aortic clamping, immediately after declamping, and 30 min after declamping. cGMP concentration was measured using YAMASA Cyclic GMP Assay Kit (Yamasa Corp., Chiba, Japan) and 8-isoprostane was measured using Cayman Chemical Company 8-isoprostane EIA kit (Cayman Chemical Company, Ann Arbor, MI, USA).

For determination of myocardial Ca^{2+} and residual adenosine triphosphate (ATP) levels, myocardial specimens were snap-frozen immediately after the completion of experimental protocol by cutting off the muscle around the diagonal branch; the myocardium was isolated from inside of the inner membrane. For evaluation of ischemia-reperfusion injury, we collected baseline data before CPB ($N = 8$). A portion of each specimen was immediately frozen with liquid nitrogen. After 0.1 N hydrochloric acid cooled in ice was added to the frozen specimen, the myocardial specimen was homogenized. The homogenate was centrifuged at 13,000 rpm and 4 °C for 40 min. The Ca^{2+} concentration was measured by atomic absorption spectrophotometry using a Hitachi Z 6100 analyzer (Hitachi, Ltd., Tokyo, Japan). ATP was measured by the firefly luciferin-luciferase method using a Kikkoman ATP assay kit (Kikkoman, Ltd., Tokyo, Japan).

Hemodynamic measurements

A conductance catheter tip manometer (2012-6-27-P, Alpha Medical Instruments Inc., Madero, California, USA) was inserted through the aortic valve into the left ventricle from the left carotid artery. A catheter tip manometer (811-195S/ANP534, Sentron Inc., Zoetermeer, Netherlands) was also inserted into the left ventricle to monitor continuous left ventricular pressure. The left ventricular pressure volume loop (P–V loop) was recorded using a Sigma-5 (Cardio Dynamics Inc., Zoetermeer, Netherlands).

Hemodynamic data, i.e. aortic pressure, E_{max}, which is a sensitive myocardial contractile force indicator, and Tau, which is a sensitive measure of myocardial diastolic function, were calculated

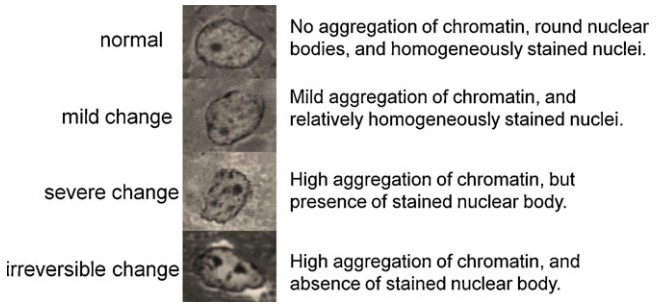

Fig. 2. Nuclear chromatin was classified according to light microscopic findings. Nuclear chromatin was observed in myocardial cells under light microscopy and classified according to four grades [normal (−), mild ischemic changes (+), severe ischemic changes (++), and irreversible ischemic changes (+++)]. (−) No aggregation of chromatin, round nuclear bodies, and homogenously stained nuclei. (+) Mild aggregation of chromatin, and relatively homogenously stained nuclei. (++) High aggregation of chromatin, but presence of stained nuclei. (+++) High aggregation of chromatin, and absence of stained nuclei.

by analyzing the *P–V* loop with Conduct PC (Cardio Dynamics Inc.). We compared E_{max} before CPB with that after CPB to evaluate contractile function. We then compared Tau before CPB with that after CPB to evaluate diastolic function. Moreover, to assess reperfusion arrhythmias, PVCs were counted during the 30 min period after reperfusion.

Histopathological studies

Another portion of myocardial specimens was examined microscopically. For microscopy, muscle specimens were fixed with a 2.5% glutaraldehyde fixative at 4 °C for 12 h. The specimens were dehydrated in ethanol and then propylene oxide, and embedded in Quetol 812 (Nissin EM Co., Ltd., Tokyo, Japan). The embedded specimens were sectioned using an Ultracut UCT ultramicrotome (Leica Microsystems, Vienna, Austria) to prepare semi-ultrathin sections and ultrathin sections. The semi-ultrathin sections were stained with toluidine blue, and observed using a light microscope to examine nuclear chromatin in myocardial cells. Nuclear chromatin was classified according to four grades (normal, mild, severe, irreversible) as in a previous study [15] to assess ischemic changes quantitatively in each treatment group (Fig. 2). We examined 400 randomly selected nuclei. The ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed using an electron microscope JEM 1200EX (JEOL Ltd., Tokyo, Japan). All microscopic examinations were performed by blinded observers.

Statistical analysis

All data are expressed as means ± standard deviation. The paired *t*-test and one factor analysis of variance were applied to test the statistical significance of differences in each group. Significant differences among the groups, if observed, were analyzed by the Scheffe method of multiple comparison, and $p < 0.05$ was considered significant.

Results

Hemodynamics

There were no significant differences in mean aortic pressure among the 4 groups: "pre CPB and post CPB" values were 78.4 ± 7.7 mmHg and 75 ± 7.6 mmHg for the control 15 min group, 79.2 ± 5.4 mmHg and 72.8 ± 4.8 mmHg for the hANP 15 min group, 80 ± 5.4 mmHg and 73 ± 6.4 mmHg for the control 30 min group, and 81.5 ± 4.4 and 76 ± 5.5 mmHg for the hANP 30 min group, respectively.

Plasma concentration of cGMP

The changes in plasma cGMP concentrations in each group are shown in Fig. 3A. After reperfusion, plasma cGMP levels were significantly increased in the hANP groups (control 15 min group 41.2 ± 11.0 pmol/mL vs. hANP 15 min group 106.5 ± 11.0 pmol/mL, $p = 0.005$; control 30 min group 36.7 ± 5.5 pmol/mL vs. hANP 30 min group 127.2 ± 19.1 pmol/mL, $p < 0.001$).

Plasma concentration of 8-isoprostane

The changes in plasma 8-isoprostane concentrations in each group are shown in Fig. 3B. Infusion of hANP significantly inhibited the increase in 8-isoprostane after declamping in the 30 min arrest group (control 30 min group 111.6 ± 13.4 vs. hANP 30 min group 53.4 ± 7.6, $p = 0.008$) and concentrations were elevated 30 min after declamping in both arrest groups (control 15 min group 102.2 ± 9.8 vs. hANP 15 min group 55.1 ± 6.5, $p = 0.049$; control 30 min group 118.6 ± 19.8 vs. hANP 30 min group 56.8 ± 10.1 $p = 0.020$).

Tissue Ca^{2+} concentration

Myocardial Ca^{2+} levels are shown in Fig. 4A. Infusion of hANP significantly inhibited the increase in myocardial

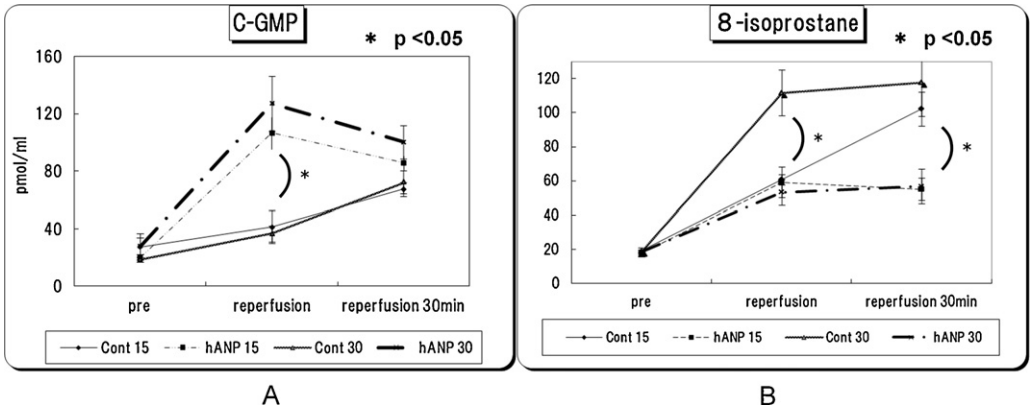

Fig. 3. Plasma concentrations of cGMP and 8-isoprostane. After reperfusion, plasma cGMP was significantly increased in the hANP groups (control 15 min group vs. hANP 15 min group, $p = 0.005$; control 30 min group vs. hANP 30 min group, $p < 0.001$). Infusion of hANP significantly inhibited the increase in 8-isoprostane after declamping in the 30 min arrest group (control 30 min group vs. hANP 30 min group, $p = 0.008$) and 30 min after declamping in each of the arrest groups (control 15 min group vs. hANP 15 min group, $p = 0.049$; control 30 min group vs. hANP 30 min group $p = 0.020$). c-GMP, cyclic guanosine monophosphate; Cont, control; hANP, human atrial natriuretic peptide.

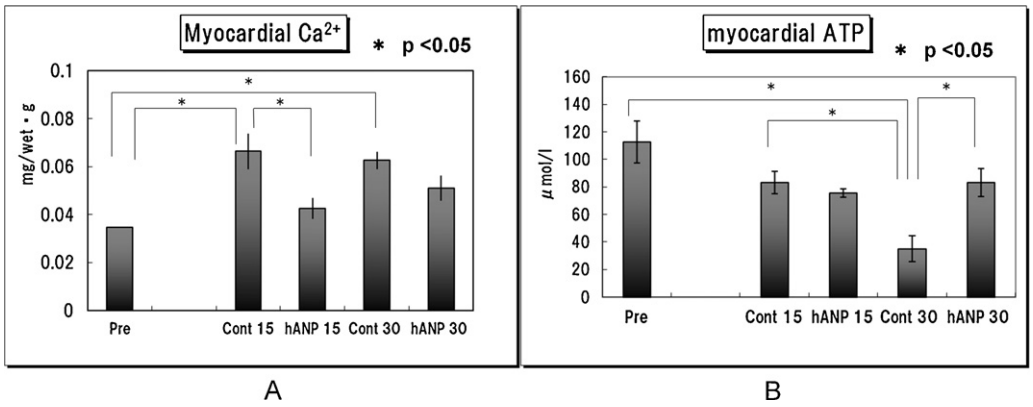

Fig. 4. Tissue concentrations of Ca^{2+} and residual ATP. After reperfusion, myocardial Ca^{2+} was significantly increased in the control groups (pre vs. control 15 min group, $p = 0.015$; pre vs. control 30 min group, $p = 0.035$). The myocardial Ca^{2+} levels in control groups were lower than those in the hANP groups (control 15 min group vs. hANP 15 min group, $p = 0.002$; control 30 min group vs. hANP 30 min group, $p = 0.110$). After reperfusion, residual ATP levels in the myocardium were significantly decreased in the control 30 min group (vs. pre, $p = 0.001$; vs. control 15 min group, $p = 0.022$; vs. hANP 30 min group, $p = 0.015$). Cont, control; hANP, human atrial natriuretic peptide.

Ca^{2+} after reperfusion. The baseline myocardial Ca^{2+} level was 0.034 ± 0.002 mg/wet g prior to CPB. After reperfusion, myocardial Ca^{2+} concentrations were significantly increased in control groups (pre vs. control 15 min group, $p = 0.015$; pre vs. control 30 min group, $p = 0.035$). Myocardial Ca^{2+} levels in control groups were lower than those in the hANP groups (control 15 min group 0.066 ± 0.0074 mg/wet g vs. hANP 15 min group 0.043 ± 0.0044 mg/wet g, $p = 0.002$; control 30 min group 0.063 ± 0.0032 mg/wet g vs. hANP 30 min group 0.051 ± 0.0043 mg/wet g, $p = 0.110$).

Tissue concentrations of residual ATP

The residual ATP levels in the myocardium are shown in Fig. 4B. After reperfusion, residual ATP levels in the myocardium were significantly decreased in the control 30 min group (pre CPB as baseline 112.9 ± 15.3 μmol/L, $p = 0.001$; control 15 min group 83.2 ± 7.9 μmol/L, $p = 0.022$; control 30 min group 35.0 ± 9.4 μmol/L, hANP 30 min group 83.3 ± 10.2 μmol/L, $p = 0.015$). Infusion of hANP significantly inhibited the decrease in the ATP level after reperfusion in the 30 min arrest group.

Cardiac catheterization

Recovery of E_{max}

E_{max} values, representing myocardial contractile force, are shown in Fig. 5A. After reperfusion, E_{max} was significantly decreased in the control 30 min group. Infusion of hANP significantly inhibited the decrease on E_{max} recovery after reperfusion in the 30 min arrest group (control 15 min group: pre 5.56 ± 0.17, post 3.9 ± 0.11, recovery of E_{max} (%) 70.17 ± 1.07% vs. hANP 15 min group: pre 5.74 ± 0.40, post 5.15 ± 0.50, recovery of E_{max} 89.24 ± 4.34%, control 30 min group: pre 4.57 ± 0.19, post 2.95 ± 0.24 (vs. pre $p = 0.049$), recovery of E_{max} 64.50 ± 4.84%, hANP 30 min group: pre 4.15 ± 0.30, post 4.73 ± 0.31 (vs. pre $p > 0.05$), recovery of E_{max} 115.05 ± 9.89%).

Changes in Tau

Tau, as a parameter of diastolic function, did not differ significantly among the groups (control 15 min group: pre 27.81 ± 0.80, post 31.46 ± 1.26, change in Tau (%) 113.3 ± 4.62% vs. hANP 15 min group: pre 27.92 ± 1.02, post 26.90 ± 1.98, change in Tau 96.5 ± 6.86%, control 30 min group: pre 33.33 ± 1.77, post 37.43 ± 1.37, change in Tau 112.6 ± 2.6%, hANP 30 min group: 34.75 ± 2.66, post 35.92 ± 4.82, change in Tau 102 ± 7.4%).

Reperfusion arrhythmias

PVCs

The number of PVCs, serving as an index of reperfusion arrhythmias are shown in Fig. 5B. Infusion of hANP significantly inhibited increases in PVC after reperfusion as compared to numbers in the control groups (control 15 min group 34.8 ± 1.8 count/30 min

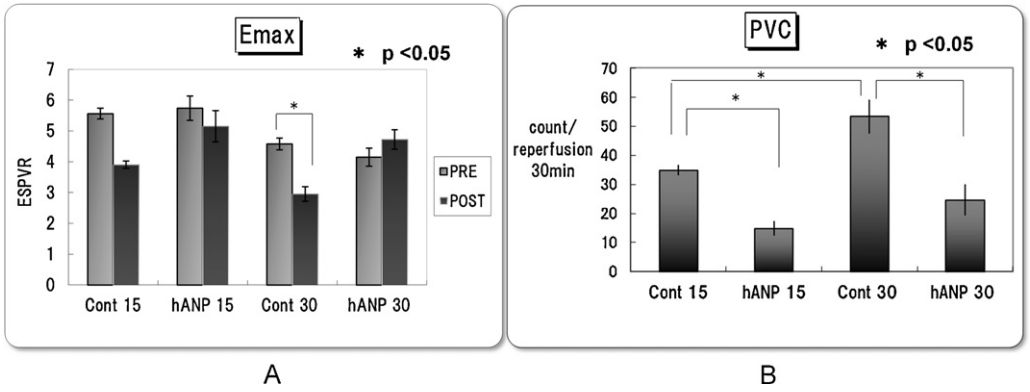

Fig. 5. Recovery of E_{max} and reperfusion arrhythmias. After reperfusion, E_{max} was significantly decreased in the control 30 min group (vs. pre $p = 0.049$). Among reperfusion arrhythmias, numbers of PVCs were significantly lower in the hANP groups than in the control groups, and the PVC count was also significantly lower in the control 15 min group than in the control 30 min group (control 15 min group vs. hANP 15 min group, $p = 0.033$; control 30 min group vs. hANP 30 min group, $p = 0.002$; control 15 min group vs. control 30 min group, $p = 0.040$). PVC, premature ventricular contraction; ESPVR, end-systolic pressure–volume relationship; Cont, control; hANP, human atrial natriuretic peptide.

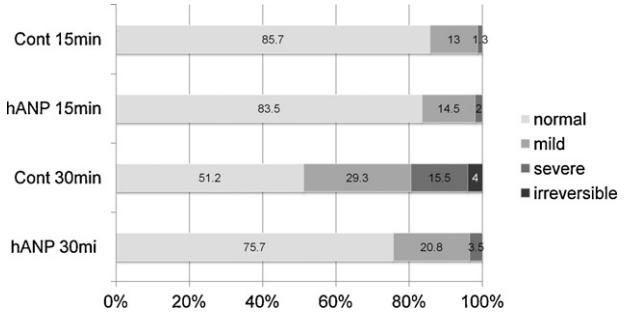

Fig. 6. Histopathological studies, ischemic changes in nuclear chromatin. There were no significant differences among the 15 min arrest groups. Severe ischemic changes were increased in the control 30 min group but changes in the hANP 30 min and 15 min groups were quite similar. There were slight but irreversible ischemic changes in the control 30 min group. Cont, control; hANP, human atrial natriuretic peptide.

vs. hANP 15 min group 14.8 ± 2.5 count/30 min $p = 0.033$, control 30 min group 53.3 ± 5.8 count/30 min vs. hANP 30 min group 24.6 ± 5.3 count/30 min $p = 0.002$).

Histopathological studies

Ischemic changes in nuclear chromatin

We used light microscopy with Toluidine Blue-staines images to examine ischemic changes in nuclear chromatin, which were classified according to four grades as in a previous study (Fig. 2) [15].

There were no significant differences between the 15 min arrest groups. The 15 min arrest groups showed mild ischemic changes but it was not irreversible. The incidence of severe ischemic changes was 1.3% (5/400) in the control 15 min group and 2.0% (8/400) in the hANP 15 min group. The incidence of severe ischemic changes was 15.5% (62/400) in the control 30 min group and 3.5% (14/400) in the hANP 30 min group. There were irreversible ischemic changes in the control 30 min group, affecting 4% of cells (16/400). Injection of hANP significantly inhibited ischemic change in the 30 min arrest group (Fig. 6).

Electron microscopic images

We evaluated the light microscopic results in greater detail, by employing electron microscopy. There were few severe ischemic changes in the 15 min arrest groups, as evidenced by normal mitochondria, myocardial fibers, and myocardial nuclei. Severe ischemic changes and irreversible ischemic changes were increased in the control 30 min group, as shown by swollen mitochondria, myocardial fibers with fuzzy contraction bands, and aggregation of chromatin around the nuclei. There were few severe ischemic changes in the hANP 30 min group, with mitochondria, myocardial fibers, and myocardial nuclei appearing essentially normal in electron microscopic images. Electron microscopy revealed a remarkable difference between the control 30 min group and the hANP 30 min groups.

Discussion

It is generally accepted that a single brief occlusion of a coronary artery (5–15 min) does not damage the myocardium in terms of necrosis or persisting contractile abnormalities. However, a single brief arrest might induce reversible injury, i.e. myocardial ischemic reperfusion injury manifesting as reperfusion arrhythmias, microvascular damage, myocardial stunning, or 'reversible mechanical dysfunction', myocardial remodeling, and the triggering of apoptosis [18,19]. Prolonged myocardial ischemia can cause irreversible myocardial injury. The mechanisms of ischemic reperfusion injury are thought to involve more than 26 pathways including oxidative stress, Ca^{2+}-overload, decreasing residual ATP levels, inflammatory cytokines, neutrophils, endothelin, and so on. These various pathways may function either together or separately [20–23]. There are two main hypotheses, oxidative stress and Ca^{2+}-overload, which have been proposed to explain the pathogenesis of ischemic reperfusion injury. These two mechanisms are most likely inter-related but whether they operate simultaneously or, instead, one precedes the other is not known.

In our previous study, we demonstrated that hANP improves ischemic reperfusion injury and suggested that hANP exerts direct myocardial protective effects against myocardial ischemic reperfusion injury associated with using cardioplegia during CPB [15,16]. We did not evaluate the effect on left ventricular function and arrhythmias after ischemic reperfusion injury in the previous study.

Possible beneficial effects of hANP on ischemic reperfusion injury

The present study showed histopathologically that the control 15 min arrest group developed reversible myocardial injury (slight ischemic change). The control 30 min arrest group began to show severe ischemic changes as irreversible myocardial injury in histopathological studies. In this group, the prevalence of arrhythmias after reperfusion increased and the myocardial contractility after reperfusion (E_{max}) was decreased.

In the present study, a "hANP shot", i.e. intravenous administration of hANP, significantly inhibited reperfusion arrhythmias with 15 min arrest. With prolonged (30 min) arrest, administration of hANP inhibited ischemic reperfusion injury according to histopathological studies, decreased reperfusion arrhythmias, and improved myocardial contractility as reflected by E_{max}. These findings suggested that hANP exerts a direct improved cardiac contraction and reduced reperfusion arrhythmias after cardiac arrest during CPB without cardioplegia. In other words, the hANP shot allowed prolonged aortic cross-clamping time and inhibited ischemic reperfusion injury associated with cardiac arrest without cardioplegia.

We showed plasma concentrations of c-GMP to be increased after hANP shot administration under ischemic conditions and that myocardial Ca^{2+} concentrations were significantly decreased after reperfusion. Based on the results of the present study, we speculate that hANP leads to the synthesis of cGMP and inhibits increases in Ca^{2+} concentrations via activation of c-GMP-dependent protein kinase (cGK), and Ca^{2+} is then released from cells.

Oxidative stress injures myocardial cell membranes and mitochondria [22]. In our present study, concentrations of 8-isoprostane in plasma samples collected from the coronary sinus, a marker of cardiac oxidative stress, were decreased after hANP shot administration under ischemic conditions. Also mitochondria in electron microscopy were preserved and ischemic changes in nuclear chromatin were inhibited by hANP shot. Oxidative stress may result in cellular defects including depression of sarcolemmal (SL) Ca^{2+}-pump ATPase and Na^{+}–K^{+} ATPase activities. Depression of the Ca^{2+}-regulatory mechanism by reactive oxygen species (ROS) ultimately results in Ca^{2+} overload. On the other hand, an increase in myocardial Ca^{2+} concentrations during ischemia induces the conversion of xanthine dehydrogenase to xanthine oxidase and subsequently results in the generation of superoxide radicals. Kiemer et al. showed hANP to inhibit activation of iNOS through nuclear factor-kappa B (NF-κB) [24]. Kathrin et al. showed c-GMP to inhibit NF-κB and tumor necrosis factor-α (TNF-α) [25]. These results suggest that hANP inhibits cardiac oxidative stress after ischemic reperfusion through NF-κB. Oxidative stress and Ca^{2+} overload seem to occur together. In this study, hANP maintained myocardial residual ATP after ischemic reperfusion. Mitochondrial injury after ischemic reperfusion reduces ATP synthetic capacity.

It has been reported that an increase in the c-GMP concentration leads to a decrease in oxygen consumption in the myocardium [26]. It is thus reasonable to speculate that inhibition of Ca^{2+} overload and oxidative stress with administration of hANP preserves ATP synthetic capacity.

hANP reportedly inhibits inflammatory cytokines such as TNF-α and modulates neutrophil functions, as well as exerting protective effects against neutrophils inducing endothelial cytotoxicity in ischemic reperfusion injury [27].

In other words, hANP may be an ideal medication for the prevention of ischemic reperfusion injury, which occurs via several pathways.

Administering hANP shot may facilitate weaning from CPB and avoid postoperative low out-put syndromes. Treatment with hANP is anticipated to provide cardiac protection during prolonged open heart surgery and cardiac transplantation. Based on the satisfactory results obtained in our present experiment, clinical application was approved by the Ethics Review Board of Nihon University Itabashi Hospital and a clinical trial of the "hANP shot" has already begun. To date, satisfactory results have been obtained.

This study employed an acute phase model and thus we could not assess chronic phase results. We hypothesize that treatment with hANP in the acute phase during CPB might prove more effective in chronic phase treatment of postoperative heart failure.

In this study we evaluated "hANP shot" without cardioplegia. Various methods for cardiac protection have been reported in the world. Therefore, we should evaluate the effect of and problems with "hANP shot" for cardiac protection in the future.

Conclusions

The present study showed administration of hANP to inhibit Ca^{2+} overload and oxidative stress associated with ischemic reperfusion injury.

The hANP shot exerts a direct myocardial protective effect against myocardial ischemic reperfusion injury without cardioplegia, inhibits severe ischemic change, as shown by histopathological studies, improves cardiac contraction, and reduces reperfusion arrhythmias after cardiac arrest during CPB.

The hANP shot allows the aortic cross-clamping time to be prolonged and inhibits so-called stunning of the myocardium associated with cardiac surgery.

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