

# Experimental Study on Myocardial Protection by Adjunct Use of Carperitide (hANP) in Cardiac Surgery

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**Background:** In recent years, various beneficial roles of human atrial natriuretic peptide (hANP) have been demonstrated in the internal medicine and surgical fields. However, direct myocardial protection by hANP against myocardial ischemic reperfusion injury has been rarely investigated. Thus, we investigated it from aspects of cardiac surgery.

**Methods:** Twenty-four pigs underwent extracorporeal circulation and were divided into three groups: control group (treated with only cardioplegic solution after aorta clamping; cardioplegic arrest for 30 minutes followed by reperfusion for 60 minutes); low dose group (treated with cardioplegic solution and ANP (25  $\mu$ g)); and high dose group (treated with cardioplegic solution and ANP (100  $\mu$ g)). Blood and myocardial cGMP, myocardial Ca and ATP concentration were determined. Histological examinations were performed using an electron microscope.

**Results:** Blood and myocardial cGMP and myocardial ATP levels were significantly higher in the hANP treatment groups than the control group. Myocardial Ca concentrations were significantly lower in the hANP treatment groups than the control group. In electron microscopy, ischemic reperfusion injury was rarely observed in the hANP treatment groups.

**Conclusion:** The study demonstrated that hANP improves ischemic reperfusion injury and suggested that hANP exerts direct myocardial protection against myocardial injury associated with cardiac surgery (cardioplegic arrest while cardiopulmonary bypass). (*Ann Thorac Cardiovasc Surg* 2005; 11: 12–20)

**Key words:** atrial natriuretic peptide (ANP), carperitide (hANP), ischemic reperfusion injury, myocardial protection

## Introduction

Atrial natriuretic peptide (ANP) was isolated and identified in 1984 by Kanagawa and Matsuo.<sup>1)</sup> It is predominantly synthesized in the atrium, and involved in the regulation of body fluid and circulation. An ANP preparation, carperitide (human ANP: hANP) was developed, and has been mainly used for treatment of heart failure because it improves preload and afterload by diuresis<sup>2,3)</sup> and vasodilatation.<sup>4-6)</sup> In cardiac surgery performed at our depart-

ment, we have experienced satisfactory results by overcoming the disadvantages of cardiopulmonary bypass (CPB), such as complications including decreased urinary output, decreased ANP, and third-space fluid shift, by means of transvenous infusion of hANP at a low dose (0.02-0.05  $\gamma$ ) simultaneously with the start of the CPB.<sup>7)</sup> In addition, it has been recognized that hANP decreases the demand of furosemide after surgery, and inhibits the loss of potassium, deteriorated electrolyte balance and development of arrhythmias during unstable conditions after surgery. Today, hANP has become one of the indispensable drugs for peri- and postoperative management.

Recent studies demonstrated that hANP exerts various actions. It has been reported that hANP inhibits volume overload-related factors and vasopressing factors (sympathetic nervous system, renin-angiotensin system, endothelin-I, inflammatory cytokines, TNF- $\alpha$ , and IL-6)

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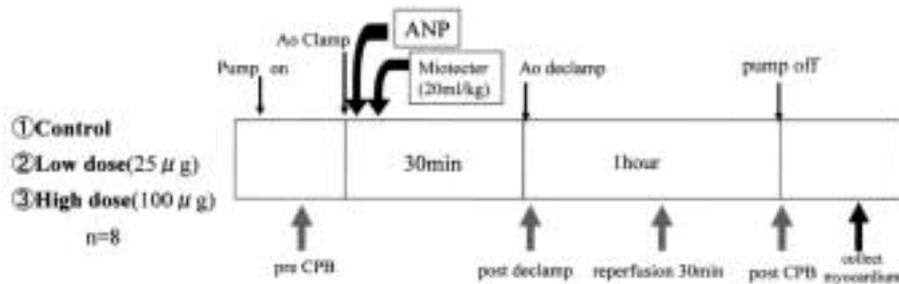


Fig. 1. Experimental method and group.

called cardiotoxic factors.<sup>8,9</sup>) It has also been reported that hANP exerts coronary vasodilatation when administered into the coronary artery during percutaneous transluminal coronary angioplasty (PTCA), and inhibits myocardial remodeling in remote areas after PTCA.<sup>10</sup> These studies suggest that ANP exerts myocardial protection. In cardiac surgery, it is indispensable. Myocardial protection may be enhanced by directly administering hANP into the heart in addition to a cardioplegic solution.

The present study investigated whether ANP exerts direct myocardial protection in a cardiac surgery model in the pig heart (cardioplegic arrest while CPB followed by reperfusion), and whether “hANP assisted cardioplegia” is feasible.

## Materials and Methods

Twenty-four male pigs (weight  $39.4 \pm 3.3$  kg) were used for the experiments. After anesthetic induction with intramuscular administration of pentobarbital (20 mg/kg) and ketamine hydrochloride (10 mg/kg), anesthesia was maintained with continuous intravenous infusion of ketamine hydrochloride (1 mg/kg/hr). An arterial line was inserted into the right common carotid artery and a Swan-Ganz catheter was introduced into the right external jugular vein for monitoring of arterial pressure, central venous pressure and pulmonary arterial pressure. Limb lead electrocardiogram (ECG) was also monitored. After endotracheal intubation, animals were ventilated with a tidal volume of 10 to 15 mL/kg and a rate of 20 to 25 breaths/min using a respirator (Servo 900E, Siemens-Elema AB, Solna, Sweden) maintaining percutaneous oxygen saturation at 95-100%. 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 by median sternotomy. A cannula (TF-024L, Research Medical Inc., Utah, USA) was inserted into the right atrium for the inferior vena cava, and another cannula (A211-5.2, Stockert Instrumente GmbH,

Munich, Germany) was inserted into the ascending aorta for blood perfusion. The CPB was prepared using a gyropump (Kyocera Corp., Kyoto, Japan) and an oxygenator (HPO-20H-C, Senko Ika Kogyo (Mera), Tokyo, Japan). In addition, a cannula (Medtronic Inc., MN, USA) was inserted into the aortic root for perfusion of a cardioplegic solution and vent. CPB was started at 37°C. After the ascending aorta was clamped, a cold (4°C) cardioplegic solution (Miotecter, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was infused (20 ml/kg) via the cannula inserted into the aortic root to achieve cardioplegic arrest for 30 minutes. Thereafter, the aorta clamping was released, and reperfusion was started. After reperfusion was continued for 60 minutes, animals were weaned from CPB (Fig. 1). No inotropic drugs were applied during the experimental course. Animals were divided into three groups according to the dose of ANP: the control group (Group C) consisting of 8 animals treated with only a cardioplegic solution, Miotecter after clamping of the ascending aorta; the low dose group (Group L) consisting of 8 animals treated with ANP at a dose of 25 µg followed by Miotecter after aorta clamping; and the high dose group (Group H) consisting of 8 animals treated with ANP at a dose of 100 µg followed by Miotecter after aorta clamping (Fig. 1). ANP was administered into the aorta near to the cardioplegic aorta cannula using a syringe with a 27G needle immediately after aorta clamping. Then, infusion of the cardioplegic solution was started. Blood specimens were collected from the catheter placed in the coronary sinus at four time points, before the CPB (pre CPB), immediately after aorta declamping (start reperfusion), 30 minutes after the start of reperfusion (reperfusion 30 min), and after the completion of reperfusion (post CPB). Using the collected blood specimens, creatine kinase (CK), lactate dehydrogenase (LDH), cyclic guanosine 3',5'-monophosphate (cGMP), lactic acid, and pyruvic acid were measured. For determination of myocardial Ca, cGMP and residual ATP levels, specimens of the myocardium were collected after



**Results**

**Hemodynamics**

No significant difference was observed in aortic pressure among the three groups: “pre CPB” 103.9±19.1 mmHg for Group L, 100.9±19.6 mmHg for Group H, 98.4±23.1 mmHg for Group C. “post CPB” 84.3±11.5 mmHg for Group L, 79.1±3.5 mmHg for Group H, 80.4±10.1 mmHg for group C. Rectal temperature of pigs was 36.3±0.5°C for Group C, 36.1±0.6°C for Group H, and 36.2±0.8°C for Group L. There was no significant difference among the three groups. Myocardial temperature was 18.3±0.9°C for Group C, 19.1±0.6°C for Group H, and 18.2±0.5°C for Group L. There was no significant difference among the three groups.

**Blood chemistry**

Blood cGMP concentrations were significantly increased

in Group H compared with those in Group C: for Group H, 66.6±22.1 pmol/mL in “start reperfusion” (p<0.0090), 63.6±21 pmol/mL in “reperfusion 30 min” (p<0.0024), and 46.5±11.6 pmol/mL in “post CPB” (p<0.0111). No significant difference was observed between Group L and Group C, but cGMP concentrations showed a tendency to increase in Group L (Fig. 3).

Blood lactic acid and pyruvic acid concentrations showed that accumulation of these components tended to be inhibited by hANP dose-dependently (Group H > Group L) (Fig. 4).

**Residual tissue ATP levels**

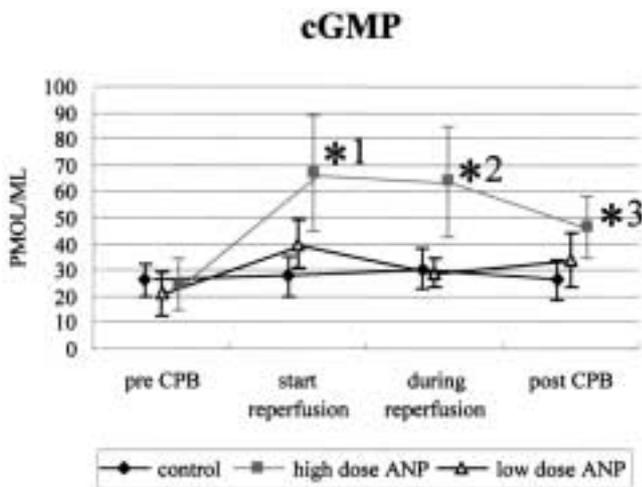
The baseline myocardial ATP level was 14.75±4.3 RLU (relative light units)/mg tissue wet weight in “pre CPB”. In “post CPB”, the residual ATP level was significantly higher in Groups H and L compared with that in Group C: 11.8±2.8 RLU/mg for Group H and 6.2±1.1 RLU/mg compared with 0.8±0.1 RLU/mg for Group C (p<0.0001, respectively) (Fig. 5).

**Tissue Ca concentrations**

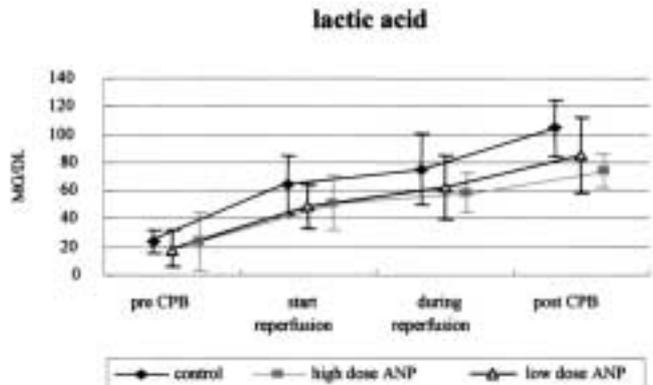
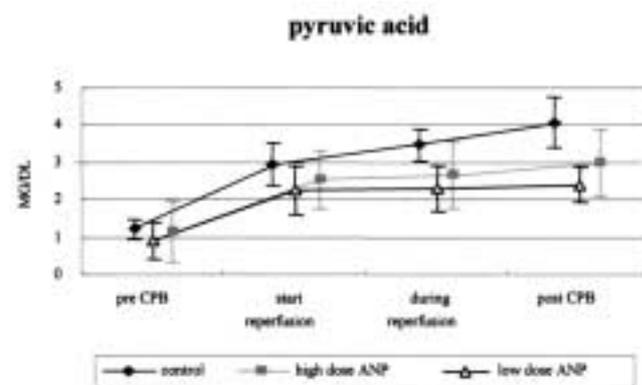
The baseline myocardial Ca concentration was 0.062±0.03 mg/g tissue wet weight in “pre CPB”. In “start reperfusion”, the myocardial Ca concentration was significantly decreased in Groups H and L compared with that in Group C, and hANP inhibited an increase in Ca concentration: 0.47±0.13 mg/g for Group H and 0.44±0.05 mg/g compared with 0.75±0.12 mg/g for Group C (p<0.0151 and 0.084, respectively) (Fig. 5).

**Tissue cGMP concentrations**

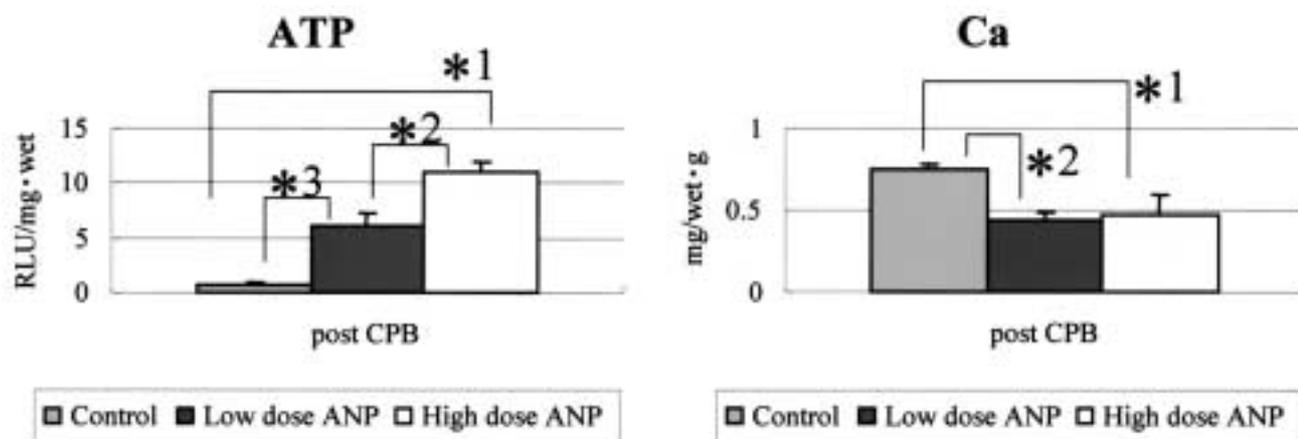
The baseline myocardial cGMP concentration was 11.0±1.84 mg/g tissue wet weight in “pre CPB”. After the completion of reperfusion, the myocardial cGMP con-



**Fig. 3.** Results of blood cGMP concentration measurements. \*1: p<0.0090, \*2: p<0.0024, \*3: p<0.0111



**Fig. 4.** Results of blood lactic acid and pyruvic acid concentration measurements.



**Fig. 5.** Results of residual myocardial ATP level and myocardial Ca concentration measurements.

\*1, \*2, \*3:  $p < 0.0001$  (ATP)

\*1:  $p < 0.084$ , \*2:  $p < 0.0151$  (Ca)

centration showed a tendency to be increased in Groups H  $9.64 \pm 2.48$  mg/g and L  $9.35 \pm 2.13$  mg/g compared with that in Group C  $6.38 \pm 0.71$  mg/g (Fig. 6).

### Electron-microscopic images

#### a. Mitochondria

Swelling of mitochondria with sparse cristae was observed in Group C. In contrast, normal mitochondria were preserved and large amounts of glycogen granules were seen in the hANP treatment groups (Fig. 7).

#### b. Myocardial fibers

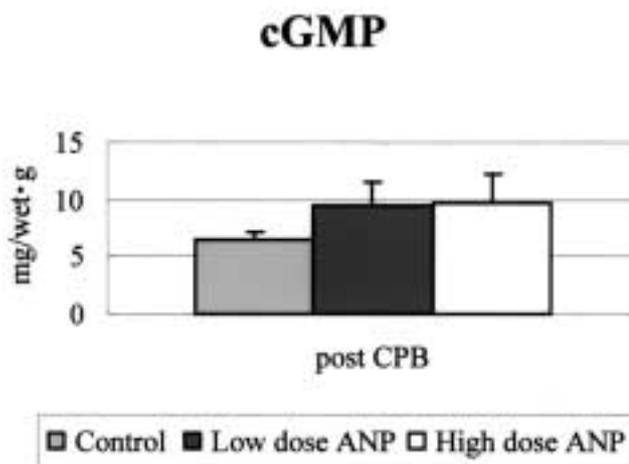
Large numbers of thick and fuzzy contraction bands were observed in Group C. In contrast, normal distinct myocardial fibers were preserved in the hANP treatment groups (Fig. 8).

#### c. Myocardial nuclei

Aggregates of nuclear chromatin or aggregation of chromatin around the nuclei were markedly observed in Group C. However, these changes were minor in the hANP treatment groups, and the nuclei were equivalent to those in the non-ischemic myocardium (Fig. 9).

#### d. Ischemic changes in nuclear chromatin

In Group C ( $n=8$ ), the incidence of mild ischemic change (+) was 56.4% (220/390). In contrast, the incidence of normal myocardium (–) was 58.3% (287/402) and 57.1% (285/499) in Groups H ( $n=8$ ) and Group L ( $n=8$ ), respectively, and ischemic changes were inhibited in the hANP treatment groups. As all three groups were treated with a cardioplegic solution, the incidence of irreversible



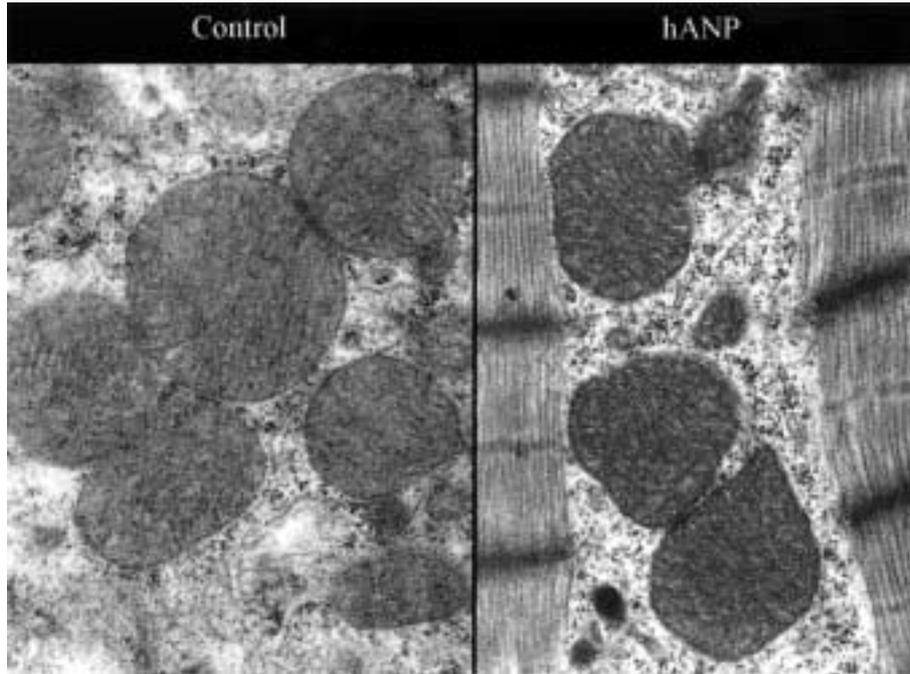
**Fig. 6.** Results of myocardial cGMP concentration measurements.

The myocardial cGMP concentration showed a tendency to be increased in Groups H and L compared with that in Group C.

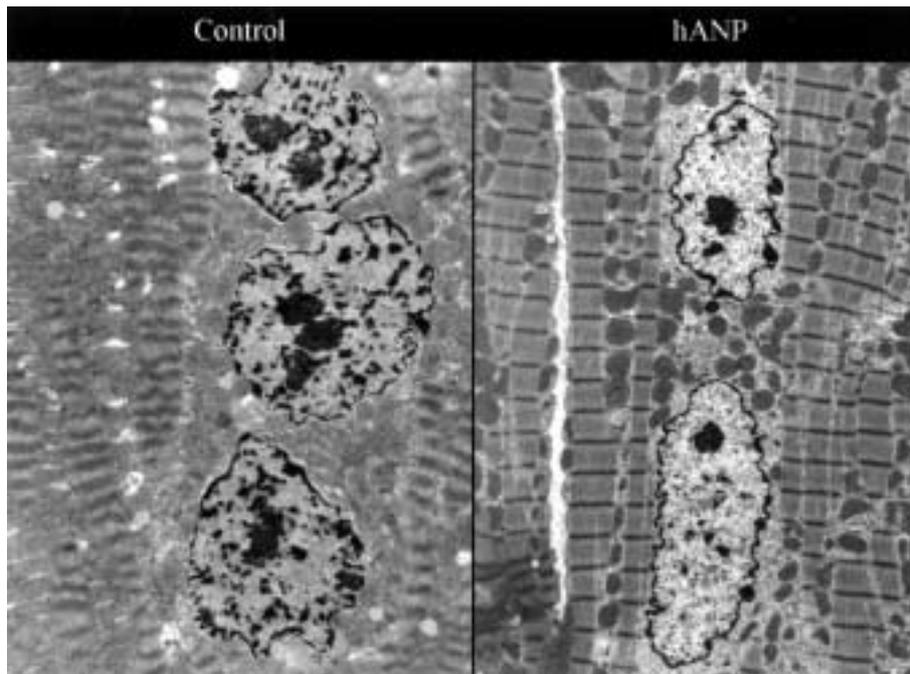
schemic change (+++) was 0%. In animals ( $n=3$ ) where reperfusion was carried out without myocardial protection, the incidence of irreversible schemic change (+++) was 60.8% (245/403) (Fig. 2).

### Discussion

In recent years, studies on myocardial protection by hANP have been occasionally reported. These studies did not investigate direct effects of ANP on the myocardium. In most of the studies, the myocardial protection is likely a secondary effect due to inhibition of cardiotoxic factors or coronary vasodilatation. Effects of hANP on ischemic reperfusion injury have been rarely reported. Padilla et



**Fig. 7.** Mitochondria in electron microscopy. Swelling of mitochondria with sparse cristae was observed in Group C. In contrast, normal mitochondria were preserved and large amounts of glycogen granules were seen in the hANP treatment groups.



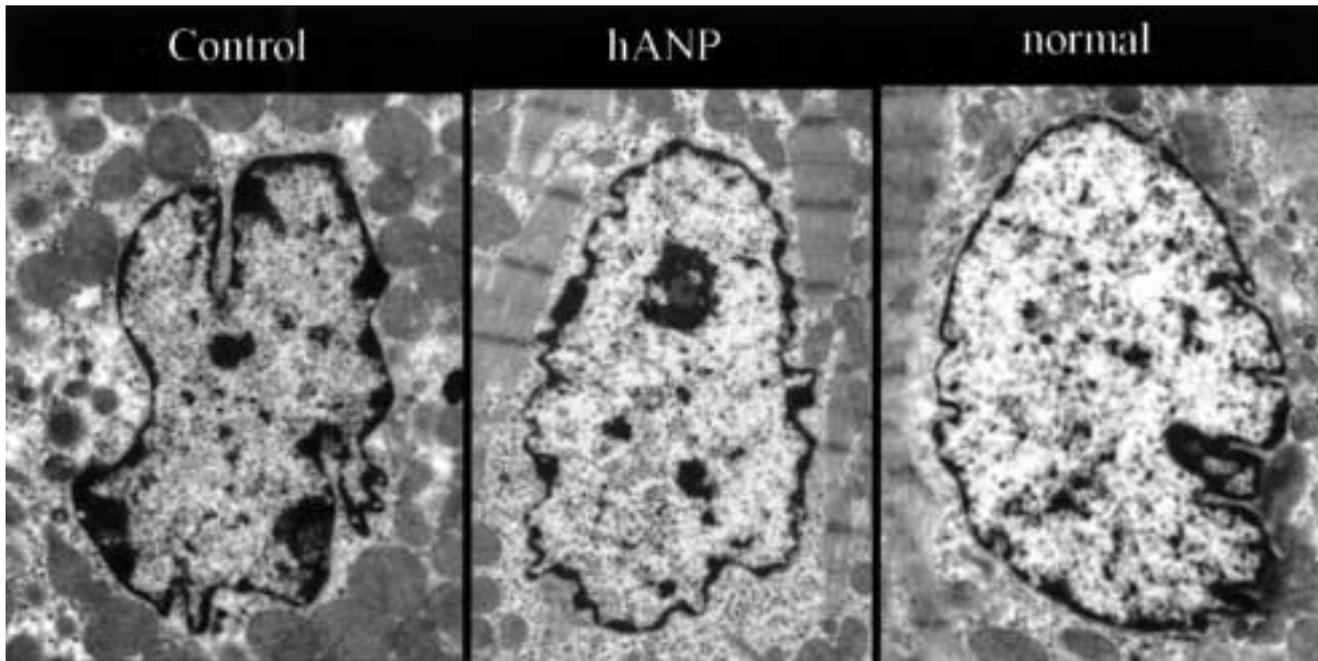
**Fig. 8.** Myocardial fibers in electron microscopy. Large numbers of thick and fuzzy contraction bands were observed in Group C. In contrast, normal distinct myocardial fibers were preserved in the hANP treatment groups.

al.<sup>11)</sup> reported that a natriuretic peptide (urodilatin, URO) significantly reduced infarct size in a pig cardiac infarction model. URO was administered by the transvenous route, to investigate the mechanism of action of URO using data from peripheral blood. In the present study, we directly administered hANP into the pig coronary artery, and blood specimens were collected from a catheter inserted into the coronary sinus. Myocardial tissue speci-

mens were also collected in order to investigate direct effects of hANP on the myocardium.

We investigated effects of hANP on ischemic reperfusion injury associated with cardiac surgery using an experimental model designed according to the process of surgical operation with aortic clamping, cardioplegic arrest, aortic declamping and reperfusion.

The myocardium is compromised by ischemia due to



**Fig. 9.** Myocardial nuclei in electron microscopy.

Aggregates of nuclear chromatin or aggregation of chromatin around the nuclei were markedly observed in Group C. However, these changes were minor in the hANP treatment groups, and the nuclei were equivalent to those in the non-ischemic myocardium.

aortic clamping during cardiac operation, resulting in impairment of aerobic energy metabolism and instead, a transient enhancement of the anaerobic glycolytic system. The glycolytic system is inactivated by accumulation of final metabolites including lactic acid. As a result, energy is already depleted when reperfusion is started because energy consumption is continued without energy supply. Various cardioplegic solutions have been developed to minimize energy consumption. Hearse et al.<sup>12)</sup> provided the following three concepts for cardioplegic solutions: 1) rapid arrest; 2) hypothermia; and 3) additional protection. Rapid arrest and hypothermia intend to achieve rapid arrest by increased potassium levels and delayed degradation of ATP by cooling the myocardium leading to inhibition of the metabolism. Additional protection mainly aims to inhibit ischemic reperfusion injury. In this study, we investigated whether hANP can contribute to additional protection, and we obtained satisfactory results.

Ischemic reperfusion injury includes the stunned myocardium<sup>13)</sup> and we hibernating myocardium<sup>14)</sup> “Stunned” means a condition after start of reperfusion that acute ischemia leads to persistent attenuation of contractility without necrosis for several hours to several days after ischemia is relieved. As a cardioplegic solution is usually used in cardiac surgery, irreversible myocardial necrosis

may not develop after aorta clamping. Therefore, the myocardium after CPB is considered to be stunned.<sup>15,16)</sup> It is a widely accepted that the potential mechanisms of development of the stunned myocardium are that Ca overload and presence of free radicals may play a role.<sup>17)</sup> It has been reported that vasodilators such as NO, CO and natriuretic peptides inhibit an increase in  $Ca^{2+}$  concentrations in the vascular smooth muscle using cGMP as the second messenger.<sup>18-20)</sup> The present study showed that myocardial and blood cGMP concentrations are increased after administration of hANP in the ischemic condition, and myocardial Ca concentrations is significantly decreased during reperfusion. Therefore, it is considered that hANP directly acts on the myocardium, and inhibits Ca overload through cGMP and improves the stunned myocardium. The mechanism that ischemic reperfusion injury develops due to Ca overload has been considered as follows. Myocardial ischemia caused by clamping of the ascending aorta leads to activation of  $Na^+/H^+$  exchange ( $H^+$  excretion) by acidosis due to anaerobic metabolism and loss of  $Na^+/K^+$ -ATPase activity due to decreased ATP, and cellular  $Na^+$  concentrations are increased. As a result, after declamping,  $Na^+/Ca^{2+}$  exchange is activated and  $Ca^{2+}$  flows in instead of  $Na^+$  excretion during reperfusion, and  $Ca^{2+}$  overload develops in the myocardium. As a re-

sult, membrane phospholipase and  $\text{Ca}^{2+}$ -dependent protease are activated, and cell membrane and cytoskeleton are broken. Thus, ischemic reperfusion injury develops. The mechanism that hANP decreases  $\text{Ca}^{2+}$  concentrations in the vascular smooth muscle has been considered as follows.<sup>19)</sup> hANP (ANP) synthesizes cGMP through guanylate cyclase (GC-A) receptors in the cell membrane. The calcium-dependent K-channel is activated through activation of cGMP-dependent protein kinase (cGK), and calcium is excreted from cells. The phospholipase C activity is inhibited, and inflow of  $\text{Ca}^{2+}$  from the  $\text{Ca}^{2+}$  channel is inhibited. Intake of calcium into the endoplasmic reticulum is increased by activation of Ca-dependent ATPase. Furthermore, outflow of calcium from the endoplasmic reticulum is inhibited by inhibition of inositol triphosphate receptors in the endoplasmic reticulum.

From the results of this study it is presumed that hANP synthesizes cGMP even in pig cardiac myocytes and that inhibits an increase in  $\text{Ca}^{2+}$  concentration.

Electron microscopy showed that mitochondria and myocardial fibers were preserved, and abnormal changes in nuclear chromatin were inhibited. These findings are considered to be caused because ischemic reperfusion injury ( $\text{Ca}^{2+}$  overload) was inhibited by the various effects of cGMP. As mitochondria are preserved, production of ATP is maintained. The increase in residual myocardial ATP is considered to be caused because ATP utilized for excretion of Ca was decreased. In addition, it has been reported that an increase in cGMP concentration leads to a decrease in oxygen consumption in the myocardium.<sup>21)</sup> It may be considered that availability of ATP was improved during reperfusion. As it has been reported that an excessive increase in cGMP concentration leads to induction of apoptosis of myocytes during reperfusion,<sup>22)</sup> the high dose of hANP was set 4 times higher than the low dose to assess the effects at overdose. However, no findings of apoptosis was observed in electron microscopy. Assessment at a further higher dose might be necessary.

For administration of hANP, other procedures such as use of a mixture of hANP with a cardioplegic solution or use of bypass might be possible. However, preliminary experiments showed that hANP is adsorbed by syringes and catheters including infusion tubes at concentration of  $10 \mu\text{g}/\text{mL}$  or lower. Thus, hANP was administered at a concentration of  $25 \mu\text{g}/\text{mL}$  into the aortic root using a syringe with a 27G needle instead of a catheter.

With regard to the doses of hANP, Ref. 10) reported that hANP exerts coronary vasodilatation and inhibits

myocardial remodeling in remote areas during PTCA when administered into the coronary artery at a dose of  $25 \mu\text{g}$ . Therefore,  $25 \mu\text{g}$  was chosen as the low dose, and  $100 \mu\text{g}$ , 4 times higher than the low dose, was chosen as the high dose. Blood chemistry data excluding cGMP showed dose-dependent effects. There was no difference in cGMP concentrations between Group L and Group C. Histological examinations showed the same effects in Groups H and L. Therefore, it is preferable to use hANP at a dose of  $100 \mu\text{g}$  to ensure stable effects though hANP was considered to inhibit myocardial injury also in Group L. With regard to the timing of administration of hANP, it has been reported that hANP inhibits irreversible myocardial injury when administered before development of ischemia for myocardial preconditioning.<sup>23)</sup> In the present study, however, hANP was administered immediately after aorta clamping followed by infusion of a cardioplegic solution. It is not appropriate to simply compare with the previous study. If myocardial protection by hANP is attributable to only inhibition of ischemic reperfusion injury, it might be more effective to administer hANP before declamping (before start of reperfusion) in consideration of the half-life of hANP. The model used in this study was a design simulating the clinical setting of cardiac surgery. By using hANP as a supplement to a cardioplegic solution instead of using hANP alone, we could obtain satisfactory results. Clinical application of hANP is expected as an efficient supplement for treatment of patients with valvular heart disease, patients with cardiac hypertrophy or enlargement, and patients for whom chronic myocardial ischemia is anticipated during cardiac operation.

## Conclusion

- 1) hANP directly acts on the heart and exerts its effects through cGMP because blood and myocardial cGMP concentrations were increased when directly administered into the coronary artery.
- 2) hANP inhibits ischemic reperfusion injury by inhibition of an increase in myocardial Ca concentrations during reperfusion. Electron microscopy showed that hANP alleviates ischemic changes in nuclei, myocardial fibers and mitochondria. hANP increases residual myocardial ATP levels.
- 3) Satisfactory results were obtained in an experimental model simulating the clinical setting for cardiac surgery. Clinical application of hANP is expected for an effective supplement in cardiac surgery.

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