J Anesth (2013) 27:901–908
DOI 10.1007/s00540-013-1643-3

ORIGINAL ARTICLE

Atrial natriuretic peptide reduces hepatic ischemia–reperfusion injury in rabbits

Takashige Yamada · Yoshifumi Kotake · Hiromasa Nagata · Junzo Takeda

Received: 24 September 2012 / Accepted: 13 May 2013 / Published online: 5 June 2013

Abstract
Purpose Atrial natriuretic peptide (ANP) has been known to be protective against hepatic ischemia/reperfusion injury. The purpose of this study was to verify the hypothesis that ANP conserves microvascular circulation and reduces ischemia–reperfusion injury in the in vivo rabbit model.
Methods With IRB approval, 30 male Japanese white rabbits under pentobarbital anesthesia were studied. These animals were randomly assigned to the following three groups ($n = 10$ each): control, ANP, and sham group. Animals in the ANP group received continuous infusion of ANP at 0.1 μg/kg/min throughout the study period. Animals in control and ANP groups underwent 90 min of partial hepatic ischemia by clamping the right hepatic artery and portal vein. Descending aortic blood flow (AoF) was monitored with a transit-time ultrasound flowmeter. Hepatic tissue microvascular blood flow (HTBF) at both right (ischemic) and left (nonischemic) lobe was intermittently evaluated with the hydrogen clearance method. After 180 min of reperfusion, hepatic injury was determined with serum AST and ALT. Galactose clearance of reperfused right lobe was also measured as an indicator of hepatic metabolic function. Histopathological change and the number of apoptotic hepatocytes were also evaluated.
Results Systemic hemodynamic data including mean arterial pressure, heart rate, and AoF did not differ among the three groups during the study period. ANP attenuated ischemia-induced right HTBF decrease. ANP also suppressed histopathological degeneration, apoptosis, and decline in galactose clearance after reperfusion.
Conclusions ANP attenuated hepatic microvascular dysfunction and hepatocyte injury after reperfusion without significant hemodynamic change.

Keywords Atrial natriuretic peptide · Ischemia–reperfusion · Liver injury · Tissue microvascular blood flow

T. Yamada (✉) · H. Nagata · J. Takeda
Department of Anesthesiology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
e-mail: yamada@e-ml.jp

Y. Kotake
Department of Anesthesiology, Toho University Ohashi Medical Center, Tokyo, Japan

Introduction

Attenuation or prevention of hepatic ischemia–reperfusion injury is an important clinical challenge in several settings such as liver surgery, liver transplantation, and shock state. The liver has several unique features in regard to the damage caused by ischemia–reperfusion compared to other organs such as myocardium and neuronal tissue. The pathophysiology of hepatic ischemia–reperfusion is characterized with derangement of sinusoidal blood flow, a significant inflammatory process caused by activated Kupffer cells, and apoptotic cell death after reperfusion [1, 2]. Several pharmacological and nonpharmacological methods to attenuate hepatic ischemia–reperfusion injury have been tested [3, 4]. Atrial natriuretic peptide (ANP) successfully protects against ischemia–reperfusion injury through various mechanisms in several vital organs and also alleviates hepatic ischemia–reperfusion injury in both in vitro and in vivo studies [5–7]. However, the effects of ANP on the aforementioned features of hepatic ischemia–reperfusion have not been fully elucidated, especially from the aspects of tissues and systemic blood flow.

The purpose of this study was to evaluate the protective effects of ANP in a rabbit model of hepatic ischemia–reperfusion injury. More specifically, we investigated the effects of ANP on the tissue microvascular blood flow, metabolic function, and morphological changes after reperfusion.

Materials and methods

With the approval of the institutional animal use and care committee, 30 male Japanese white rabbits, weighing about 2.5 kg each, were used.

Preparation

After overnight fasting, all animals were anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg), and continuous intravenous infusion of sodium pentobarbital (10 mg/kg/h) and buprenorphine (16 μg/kg/h) via the marginal ear vein. After tracheostomy, these rabbits were mechanically ventilated with 40 % oxygen at zero end-expiratory pressure (E100A; Newport Medical Instruments, Costa Mesa, CA, USA). Ventilator settings, including respiratory rate and tidal volume, were adjusted to maintain $PaCO_2$ between 30 and 45 mmHg. During the procedure, acetated Ringer's solution was infused at a rate of 10 ml/kg/h, and body temperature was maintained between 38 ° and 39 °C with a heating pad. A polyethylene tube inserted into the carotid artery was used for measurement of arterial blood pressure and drawing the blood samples.

After midline laparotomy, an ultrasonic perivascular blood flow sensor (Transonic flowprobe MA4 PSB; Transonic Systems, Ithaca, NY, USA) was attached to the descending aorta. Platinum needle electrodes of tissue microvascular blood flow measurement were inserted into the left and right lobe of the liver and a reference electrode was subcutaneously inserted in the inguinal area.

Measurements

Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored (Lifescope14; Nihon Kohden, Tokyo, Japan). Arterial blood was collected and analyzed with a blood gas analyzer (ABL-700; Radiometer A/S, Copenhagen, Denmark) at baseline, before ischemia, at end of ischemia, and at 60, 120, and 180 min after reperfusion.

Descending aortic blood flow (AoF) was monitored using a transit-time ultrasound flowmeter (T106 Flowmeter module; Transonic Systems) during the study period, and the data were stored in the recorder.

Hepatic tissue microvascular blood flow (HTBF) was measured with the hydrogen clearance method [8] at baseline, after clamping, and at 60, 120, and 180 min after reperfusion. Briefly, this method is dependent on the fact that tissue blood flow can be determined by the washout curve of inhaled hydrogen concentration. The hydrogen concentration was determined with the micro-electrical current generated on the thin platinum electrode placed in the liver parenchyma. Before the measurement, hydrogen gas was continuously inhaled by the animal at a concentration of 5 %. After stabilization, the inhalation of hydrogen gas was abruptly stopped and the washout curve of hydrogen at the liver parenchyma was determined by a specific system (MHG-D1 and UAS-108S; Unique Medical, Tokyo, Japan). HTBF was expressed in ml/min/100 g tissue.

Liver damage was determined by serum aspartate and alanine amino transferase activity (AST and ALT, respectively) measured with a commercially available assay kit (Wako Pure Chemical, Tokyo, Japan). Metabolic function of the liver was also evaluated by galactose clearance because the metabolism of galactose is completely dependent on hepatocytes [9]. To assess the function of the reperfused right lobe, vessels perfusing the left lobe were clamped before this analysis. Briefly, 0.5 mg/kg galactose dissolved in normal saline was given intravenously. Serial blood samples were collected at 5, 15, 30, 45, and 60 min after galactose injection, and the serum concentration of galactose was assayed with a commercially available kit for galactose (Wako Pure Chemical). Because galactose elimination follows first-order kinetics, its clearance was calculated from the plasma concentration curve and expressed in an elimination rate constant.

Liver tissue samples were obtained from the reperfused right lobe in the control and ANP groups at the end of the study. Each sample was fixed in 10 % buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Ten preparations from each group were microscopically analyzed, and the damage was quantitatively evaluated with the following 4-point score [10]: (0) no hepatocellular damage; (1) mild injury characterized by cytoplasmic vacuolization and focal nuclear pyknosis; (2)moderate injury with dilated sinusoids, cytosolic vacuolization, and blurring of intercellular borders; (3) moderate to severe injury with coagulation necrosis, abundant sinusoidal dilation, red blood cell extravasation into interstitium and hypereosinophilia and margination of neutrophils; and (4) severe necrosis with loss of hepatic architecture, hemorrhage, and neutrophil infiltration. Additional sections were stained with a commercially available TUNEL (TdT-mediated dUTP-X nick-end labeling) assay kit (In Situ Cell Death Detection Kit; F. Hoffmann-La Roche, Basel, Switzerland). The number of TUNEL-positive cells was counted in 15 randomly selected fields at 100× magnification. The total number of cells

was counted simultaneously, and then the percentage of TUNEL-positive cells was calculated. These microscopic evaluations were ratified by a pathologist who was blinded to the allocation.

Intervention

After equilibration and baseline measurement, rabbits were randomly assigned to the following three groups: ANP group, control group, and sham operated group ($n = 10$ each). The investigators were blinded to the group allocation during the preparation and also blinded to the study drugs through the experiment. Rabbits in the ANP group received continuous infusion of human ANP (Asubio Pharma, Kobe, Japan) at 0.1 μg/kg/min throughout the study period whereas animals in the control and the sham groups received the same amount of vehicle. In ANP and control groups, partial hepatic ischemia was applied by cross-clamping the right portal vein and the right hepatic artery. After 90 min of right lobe ischemia, the clamp was released and the rabbits were observed for 180 min of reperfusion period. Sham-operated animals underwent the same procedure but were not subject to right lobe ischemia. For selection of the dose of ANP and ischemia duration, a preliminary study and previous reports [7, 11] were referred to avoid systemic shock and lethal organ damages. The protocol is summarized in Fig. 1.

Statistical analysis

The results are expressed as mean ± standard deviation or median (IQR). The statistical analyses were carried out with a StatView program 5.0 (SAS Institute, Cary, NC, USA). For serially collected hemodynamic data and HTBF, repeated-measures analysis of variance (ANOVA) followed by Scheffe's post hoc test was used to evaluate differences. For the other laboratory data, one- or two-way ANOVA followed by Scheffe's post hoc test was used. The histopathological grading was analyzed by the Mann–Whitney U test. A p value less than 0.05 was considered statistically significant.

Results

Systemic hemodynamics

Each animal successfully completed the whole study protocol.

MAP, HR, and AoF are summarized in Fig. 2 and results of blood gas analysis are summarized in Table 1. There was no difference in MAP, HR, and AoF in the three groups throughout the study period. These parameters were not affected by occlusion or reperfusion of the right hepatic artery and portal vein. Animals in the ANP group showed slightly but statistically significant increase of blood lactate concentration after reperfusion compared to the sham-operated group.

Hepatic tissue microvascular blood flow

HTBF measured with hydrogen clearance method is summarized in Fig. 3. Before ischemia, right HTBF was 49 ± 10, 49 ± 11, and 53 ± 12 ml/min/100 g tissue in the control, ANP, and sham groups, respectively and there was no statistical difference between these study groups. Right HTBF was significantly decreased to 24 ± 9 ml/min/100 g tissue immediately after ischemia in the control group. Right HTBF of the ANP group decreased to 32 ± 6 ml/min/100 g tissue immediately after ischemia but demonstrated an increasing tendency afterward. Right HTBF of the ANP group was significantly higher than that of the control group at 180 min after reperfusion.

In the nonischemic left lobe, there is no significant difference in HTBF in the three groups.

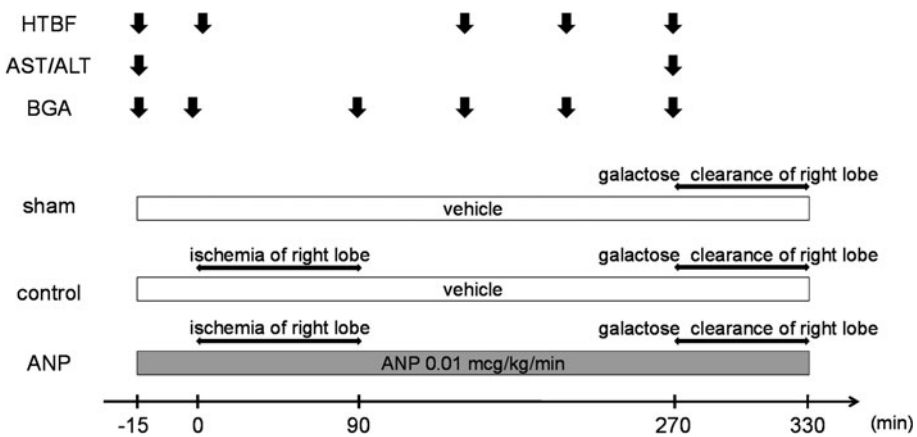

Fig. 1 Study protocol. Control and atrial natriuretic peptide (ANP) groups underwent 90 min of right lobe ischemia. The ANP group received continuous ANP infusion. After 180 min of reperfusion, all three groups underwent galactose clearance measurement. *Arrows* indicate measurement of hepatic tissue blood flow (HTFB), serum transaminase, and blood gas sampling

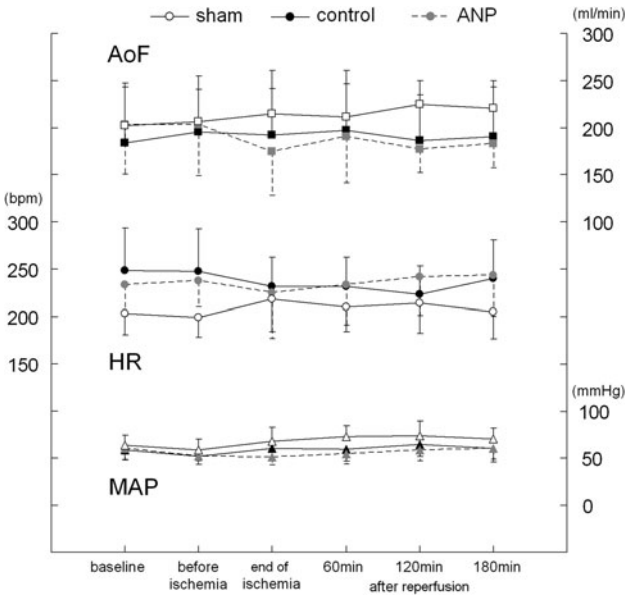

Fig. 2 Hemodynamic data. Time course of descending aortic blood flow (*upper panel*), heart rate (*middle panel*), and mean arterial pressure (*lower panel*) are summarized. Data are expressed as mean ± SD. *Blank symbols, filled symbols*, and *shaded symbols* denote sham, control, and ANP group, respectively. *AoF* descending aortic blood flow, *HR* heart rate, *MAP* mean arterial pressure

Hepatocyte damage and function

Serum AST and ALT levels increased markedly in the control and ANP groups after 180 min of reperfusion. ANP treatment significantly attenuated the increase of transaminase levels after reperfusion compared to vehicle (Fig. 4). Galactose clearance of the reperfused right lobe determined 180 min after reperfusion is summarized in Fig. 5. The elimination rate constants in the control, ANP, and sham groups were 0.0135 ± 0.0049, 0.0197 ± 0.0064, and 0.0243 ± 0.0070, respectively. Animals in the control group and in the ANP group showed reduced galactose clearance compared to the sham group, but clearance in the ANP group was significantly higher than that of the control group.

Histopathological changes

Extensive areas of cell swelling and vacuolization around the central vein were observed in the control group at the end of the study. In contrast, only mild cell swelling was seen in animals that received ANP (Fig. 6). The grading score of ANP group [0.5 (0.5–1)] was significantly lower than the control group [1.5 (1, 2)].

Table 1 Blood gas data

	Baseline	Before ischemia	End of ischemia	60 min after reperfusion	120 min after reperfusion	180 min after reperfusion
pH						
Sham	7.42 ± 0.06	7.41 ± 0.06	7.39 ± 0.06	7.38 ± 0.07	7.41 ± 0.06	7.42 ± 0.03
Control	7.43 ± 0.03	7.42 ± 0.02	7.42 ± 0.04	7.40 ± 0.04	7.43 ± 0.05	7.45 ± 0.04
ANP	7.40 ± 0.04	7.36 ± 0.08	7.38 ± 0.07	7.37 ± 0.03	7.40 ± 0.03	7.38 ± 0.06
$PaCO_2$ (mmHg)						
Sham	34.5 ± 6.2	34.8 ± 5.6	38.0 ± 6.9	41.0 ± 6.8	38.3 ± 6.6	40.0 ± 5.5
Control	35.0 ± 3.7	36.1 ± 2.5	36.7 ± 2.9	37.6 ± 4.3	36.1 ± 4.8	35.7 ± 2.6
ANP	34.8 ± 4.7	37.8 ± 7.8	34.8 ± 5.8	35.0 ± 4.3	35.8 ± 4.1	38.2 ± 4.5
PaO_2 (mmHg)						
Sham	203 ± 22	206 ± 23	198 ± 23	192 ± 21	192 ± 22	190 ± 20
Control	191 ± 13	187 ± 11	181 ± 13	188 ± 13	181 ± 18	189 ± 14
ANP	196 ± 19	187 ± 17	193 ± 15	193 ± 17	191 ± 17	187 ± 27
BE (mEq/l)						
Sham	−2.7 ± 1.6	−2.4 ± 2.3	−1.7 ± 2.5	−1.2 ± 2.6	−0.6 ± 2.0	0.9 ± 2.2
Control	−2.7 ± 1.8	−2.0 ± 2.5	−1.6 ± 1.7	−2.0 ± 1.3	−0.6 ± 1.3	−0.2 ± 1.3
ANP	−2.8 ± 2.9	−3.5 ± 1.9	−4.1 ± 2.1	−2.8 ± 2.1	−3.3 ± 2.4	−2.1 ± 2.6
Lactate (mM/l)						
Sham	1.7 ± 0.4	1.3 ± 0.5	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.5	1.4 ± 0.5
Control	2.0 ± 0.7	1.7 ± 0.4	1.5 ± 0.5	1.6 ± 0.6	2.0 ± 0.8	2.3 ± 0.9
ANP	2.0 ± 1.0	1.9 ± 0.9	2.5 ± 1.2[a]	2.6 ± 1.1[a]	2.5 ± 1.2[a]	2.7 ± 1.1[a]

Data are expressed as mean ± SD. $n = 10$ for each group

ANP atrial natriuretic peptide

[a] Statistically significant compared with sham group

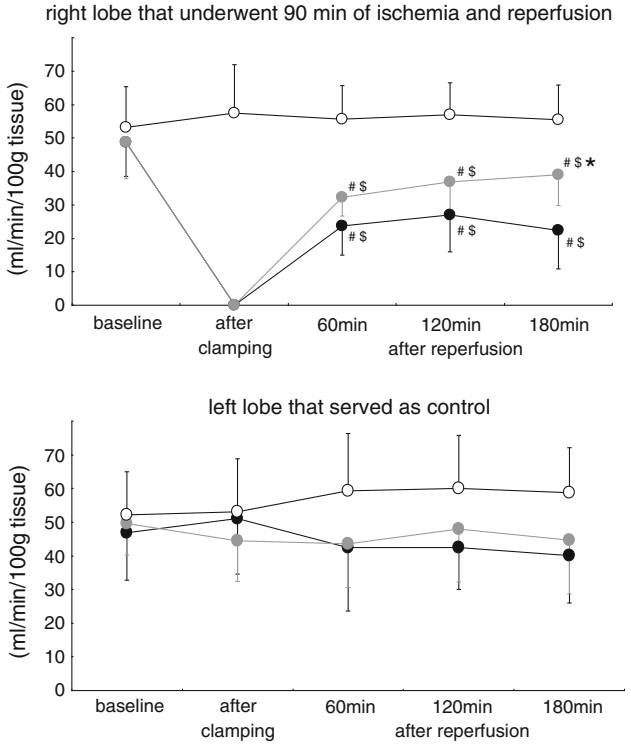

Fig. 3 Hepatic tissue microvascular blood flow. Time course of hepatic microvascular blood flow is summarized in this graph. *Upper panel* shows microvascular blood flow of right lobe that underwent 90 min of ischemia. *Lower panel* shows microvascular blood flow of left lobe that served as a control. Data are expressed as mean ± SD. *Blank symbols*, *filled symbols*, and *shaded symbols* denote sham, control, and ANP group, respectively. $^{\#}p < 0.05$ versus baseline, $^{\$}p < 0.05$ versus sham, $^{*}p < 0.05$ versus control

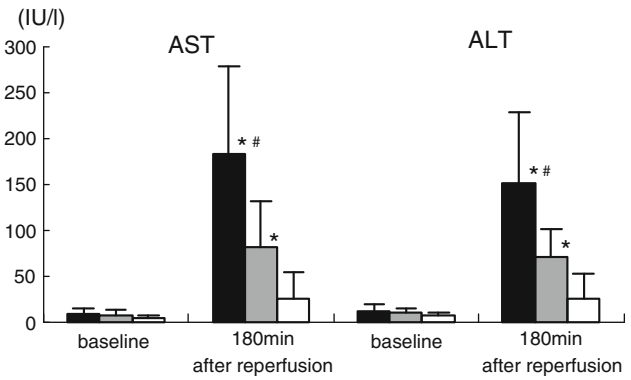

Fig. 4 Serum aspartate transaminase (AST) (*left panel*) and serum alanine transaminase (ALT) (*right panel*) at baseline and 180 min after reperfusion. Data are expressed as mean ± SD. *Blank bars*, *filled bars*, and *shaded bars* denote sham, control, and ANP group, respectively. The AST and ALT values at 180 min after reperfusion were 183 ± 96 and 153 ± 77 IU/l in the control group and 82 ± 51 and 72 ± 29 IU/l in the ANP group, respectively. $^{*}p < 0.05$ versus sham group, $^{\#}p < 0.05$ versus ANP group

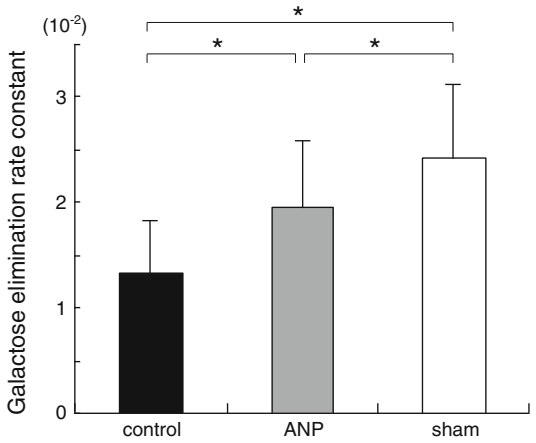

Fig. 5 Galactose clearance of postischemic right hepatic lobe 180 min after reperfusion. Data are expressed as mean ± SD. *Blank bar*, *filled bar*, and *shaded bar* denote sham, control, and ANP group, respectively. $^{*}p < 0.05$

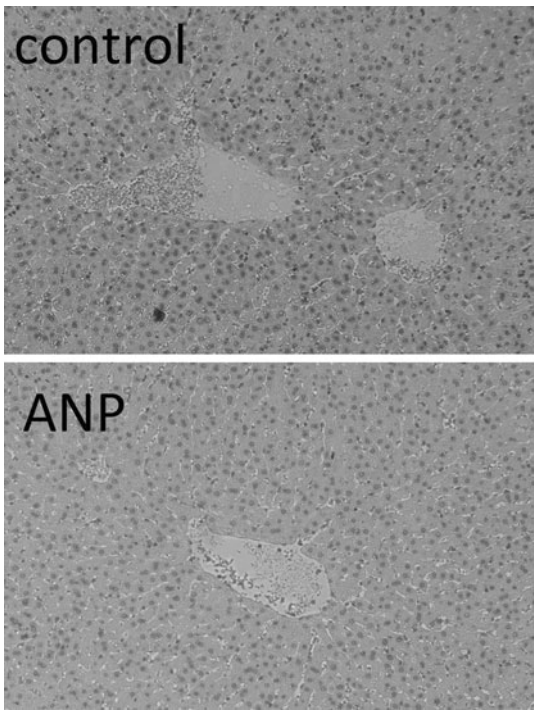

Fig. 6 Hematoxylin and eosin staining of representative liver sections from rabbits 240 min after reperfusion. Prominent necrosis, vacuolization, and sinusoidal congestion were observed in the control group (*upper panel*). These degenerative changes were attenuated in the ANP group (*lower panel*)

The percentage of TUNEL-positive cells was 29.6 ± 20.3 and 6.0 ± 7.4 % in control and ANP groups, respectively (Fig. 7). There is a statistical difference in the percentages between the control group and the ANP group.

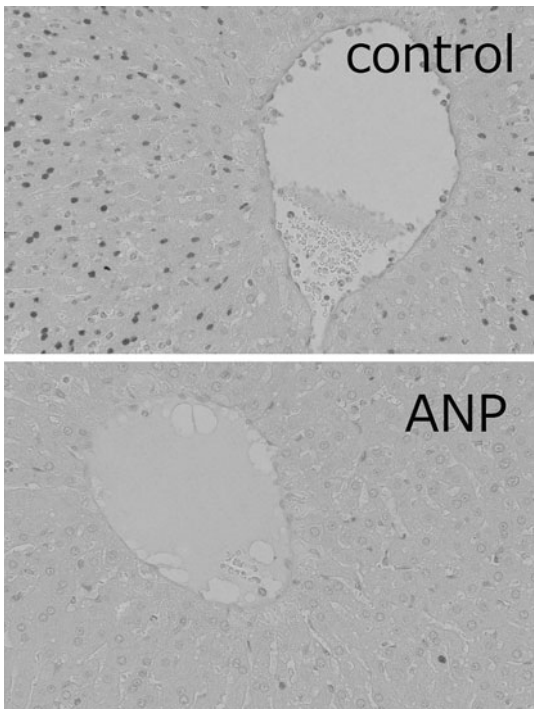

Fig. 7 TUNEL staining of representative liver sections from rabbits 240 min after reperfusion. Many more nuclear-labeled TUNEL-positive cells were observed in the control group (*upper panel*) than in the ANP group (*lower panel*)

Discussion

In this study, we demonstrated that continuous infusion of ANP throughout the ischemic and reperfusion phase significantly attenuated liver injury. More specifically, ANP attenuated the decrease of HTBF and galactose clearance after reperfusion. ANP also attenuated increase of transaminases, histopathological degradation, and apoptosis after reperfusion.

ANP is a hormone that belongs to the natriuretic peptide family, which includes a number of peptides acting as neurotransmitters or hormones [12]. Its pharmacological actions include natriuresis, diuresis, and vasodilation, and ANP acts as an endogenous producer of cyclic GMP and supplements the action of nitric oxide (NO). It is widely accepted that NO and endothelin are important regulators of sinusoidal blood flow. In the reperfusion phase after hepatic ischemia, production of NO from the sinusoidal endothelial cells was suppressed whereas the secretion of endothelin was increased [1]. These conditions may reduce sinusoidal blood flow and possibly impair functional recovery after reperfusion.

We found that ANP continuously infused at a rate of 0.1 μg/kg/min throughout the ischemic and reperfusion period promoted the recovery of HTBF after reperfusion. Decreased HTBF after reperfusion has been demonstrated by several in vivo studies and is known as the "no-reflow phenomenon." Kobayashi et al. investigated the protective effect of ANP after 120 min of total hepatic vascular exclusion in pigs under isoflurane anesthesia [7]. Using a dose of ANP identical to that of our study, they reported decreased serum transaminases and histopathological damage by ANP. They also reported ANP slightly increased HTBF 120 min after reperfusion, but the difference between ANP and vehicle did not achieve statistical significance. In our study, HTBF decreased immediately after reperfusion and remained decreased throughout the study period in the control group. In contrast, ANP gradually increased HTBF after reperfusion, and eventually HTBF in the ANP group became significantly higher compared to the control group at 180 min after reperfusion. Additionally, our study demonstrated that this beneficial effect of ANP seem to be independent of the systemic hemodynamics because other parameters, including AoF, remained comparable between the control and ANP groups.

The present study also demonstrated ANP administration attenuated the impairment of galactose clearance of the ischemic and reperfused liver tissue. Galactose is almost exclusively metabolized by the liver, and several studies demonstrated decreased galactose elimination capacity is associated with poor outcome [13–15]. We believe that the results of galactose clearance can be interpreted as the same way as galactose elimination capacity because these two methods evaluate basically identical aspects of liver function. It is not readily known whether this protective effect is caused by maintenance microvascular blood flow, attenuation of hepatocyte necrosis and apoptosis, or other factors. The cause of this protective effect is likely multifactorial and warrants further study. Blood lactate concentration was slightly but significantly increased in the ANP group compared to sham group, despite the increased HTBF. Although the underlying mechanism of this finding is not readily known, we assume this finding has little if any significance compared to the improvement of galactose clearance with ANP infusion.

Ischemia and reperfusion activates Kupffer cells and the subsequent inflammatory response contributes to the injury via a neutrophil-dependent mechanism [16–19]. Tumor necrosis factor (TNF)-α released during the inflammatory process also triggers hepatic dysfunction by promoting apoptosis [16, 18, 20].

In the present study, ANP attenuated pathological changes including cell swelling, vacuolization, and apoptosis. Apoptosis plays an important role in hepatic dysfunction after ischemia–reperfusion [21, 22]. Gerwig et al. [6] demonstrated that ANP reduced the TUNEL-positive hepatocytes by 37 % after 24 h of cold ischemia in rat liver but reported ANP has no effects on the number of

TUNEL-positive endothelial cells. We also found significant reduction of TUNEL-positive cells with ANP in examination without distinction of cell type. However, the large standard deviation makes qualitative comparison of the effects of ANP on any cell apoptosis difficult. Furthermore, the apoptotic change is highly dependent on the duration of the ischemic and reperfusion periods [23]. Thus, our finding that ANP reduces apoptosis should be interpreted with caution.

This study has several clinical implications. First, ANP demonstrated the protective effect with clinically relevant doses. Especially, we found it advantageous that ANP demonstrated protective effects without any hemodynamic derangement. Although the optimal dosing of ANP for humans remains to be determined, we assume a similar dose range used in the successful human study may be sufficient to produce clinically relevant protection [24, 25]. Second, our study demonstrated the metabolic function of hepatocyte almost normalized 3 h after reperfusion in the ANP group. Most previous studies used serum transaminases as an index of hepatocyte damage, and functional derangement after ischemia–reperfusion has been rarely evaluated. Preservation of the metabolic function of intravenous anesthetics, neuromuscular blocking agents, and opioids plays a crucial role in anesthetic management. From these perspectives, it is reasonable to assume that ANP preserves normal metabolism of various drugs used during perioperative period in cases undergoing hepatic ischemia and reperfusion. We believe that this possibility warrants further investigations.

Obviously, this study has several limitations. First, the underlying mechanisms of this protective effect are not readily known from this study. There are several possibilities that may account for the protective effects of ANP in hepatic ischemia–reperfusion other than maintaining tissue microvascular blood flow and preventing necrosis and apoptosis. ANP has a potential of improving sinusoidal perfusion through increased cyclic GMP. Additionally, inflammatory process involving Kupffer cell activation and release of inflammatory cytokines may play an important role in the pathophysiology of ischemia–reperfusion injury. Several investigations demonstrated the protective effects of ANP by suppressing Kupffer cell activation and subsequent inflammatory response [26–29]. This possibility definitely warrants further investigation. Second, the optimal dose of ANP cannot be demonstrated from this study. A higher dose of ANP may further increase HTBF but may decrease systemic blood pressure and aggravate peripheral tissue perfusion. Third, this study only evaluated the short-term effects of ischemia–reperfusion injury and the protective effects of ANP. ANP is also reported to inhibit stellate cell activation and protect hepatocytes by suppressing fibrosis in injured liver [30]. Whether ANP promotes beneficial effects in the long term after hepatic ischemia–reperfusion remains to be evaluated. Despite these limitations, this in vivo animal study provided a feasible pharmacological measure to attenuate hepatic ischemia–reperfusion injury.

In conclusion, ANP at a clinically relevant dose protects hepatocytes and preserves metabolic function after 90 min of hepatic ischemia–reperfusion in rabbits. ANP exerted protection possibly by preserving tissue microvascular blood flow and attenuating morphological damage and apoptosis after reperfusion.

Acknowledgments The authors give their heartfelt thanks to Kazunori Kikuchi, M.D., Department of Pathology, Tsukuba Medical Center, who provided carefully considered feedback and valuable comments. The authors also acknowledge Michiko Yamamoto, B.A., for her technical assistance. This work was supported by The Ministry of Education, Culture, Sports, Science and Technology: Grant-in-Aid for Young Scientists (Grant number 19791088).

Conflict of interest The authors have no conflict of interest.

References

1. Vollmar B, Menger MD. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. Physiol Rev. 2009;89:1269–339.
2. Klune JR, Tsung A. Molecular biology of liver ischemia/reperfusion injury: established mechanisms and recent advancements. Surg Clin N Am. 2010;90:665–77.
3. Clavien PA, Selzner M, Rudiger HA, Graf R, Kadry Z, Rousson V, Jochum W. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Ann Surg. 2003;238:843–50.
4. Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhan M, Jochum W, Spahn DR, Graf R, Clavien PA. A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. Ann Surg. 2008;248:909–18.
5. Cottart CH, Nivet-Antoine V, Do L, Al-Massarani G, Descamps G, Xavier-Galen F, Clot JP. Hepatic cytoprotection by nitric oxide and the cGMP pathway after ischaemia-reperfusion in the rat. Nitric Oxide. 2003;9:57–63.
6. Gerwig T, Meissner H, Bilzer M, Kiemer AK, Arnholdt H, Vollmar AM, Gerbes AL. Atrial natriuretic peptide preconditioning protects against hepatic preservation injury by attenuating necrotic and apoptotic cell death. J Hepatol. 2003;39:341–8.
7. Kobayashi K, Oshima K, Muraoka M, Akao T, Totsuka O, Shimizu H, Sato H, Tanaka K, Konno K, Matsumoto K, Takeyoshi I. Effect of atrial natriuretic peptide on ischemia–reperfusion injury in a porcine total hepatic vascular exclusion model. World J Gastroenterol. 2007;13:3487–92.
8. Young W. H2 clearance measurement of blood flow: a review of technique and polarographic principles. Stroke. 1980;11:552–64.
9. Hoekstra LT, de Graaf W, Nibourg GA, Heger M, Bennink RJ, Stieger B, van Gulik TM. Physiological and biochemical basis of clinical liver function tests: a review. Ann Surg. 2013;257:27–36.
10. Li W, Meng Z, Liu Y, Patel RP, Lang JD. The hepatoprotective effect of sodium nitrite on cold ischemia–reperfusion injury. J Transplant. 2012. doi:10.1155/2012/635179.

11. Kallaras K, Babas G, Stergiou-Michailidou V, Karamouzis M, Zaraboukas T. Atrial natriuretic peptide decreases aorta stiffness in cholesterol-fed anesthetized rabbits. Pharmacol Res. 2009;60:324–31.
12. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med. 1998;339:321–8.
13. Merkel C, Marchesini G, Fabbri A, Bianco S, Bianchi G, Enzo E, Sacerdoti D, Zoli M, Gatta A. The course of galactose elimination capacity in patients with alcoholic cirrhosis: possible use as a surrogate marker for death. Hepatology. 1996;24:820–3.
14. Garello E, Battista S, Bar F, Niro GA, Cappello N, Rizzetto M, Molino G. Evaluation of hepatic function in liver cirrhosis: clinical utility of galactose elimination capacity, hepatic clearance of D-sorbitol, and laboratory investigations. Dig Dis Sci. 1999;44:782–8.
15. Redaelli CA, Dufour JF, Wagner M, Schilling M, Husler J, Krahenbuhl L, Buchler MW, Reichen J. Preoperative galactose elimination capacity predicts complications and survival after hepatic resection. Ann Surg. 2002;235:77–85.
16. Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology. 2000;32:169–73.
17. Jaeschke H. Molecular mechanisms of hepatic ischemia–reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol. 2003;284:G15–26.
18. Jaeschke H. Mechanisms of liver injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia–reperfusion and other acute inflammatory conditions. Am J Physiol Gastrointest Liver Physiol. 2006;290:G1083–8.
19. Kiemer AK, Baron A, Gerbes AL, Bilzer M, Vollmar AM. The atrial natriuretic peptide as a regulator of Kupffer cell functions. Shock. 2002;17:365–71.
20. Arii S, Imamura M. Physiological role of sinusoidal endothelial cells and Kupffer cells and their implication in the pathogenesis of liver injury. J Hepatobiliary Pancreat Surg. 2000;7:40–8.
21. Teoh NC, Farrell GC. Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol. 2003;18:891–902.
22. Gujral JS, Bucci TJ, Farhood A, Jaeschke H. Mechanism of cell death during warm hepatic ischemia–reperfusion in rats: apoptosis or necrosis? Hepatology. 2001;33:397–405.
23. Kohli V, Selzner M, Madden JF, Bentley RC, Clavien PA. Endothelial cell and hepatocyte deaths occur by apoptosis after ischemia–reperfusion injury in the rat liver. Transplantation. 1999;67:1099–105.
24. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T, Seguchi O, Myoishi M, Minamino T, Ohara T, Nagai Y, Nanto S, Watanabe K, Fukuzawa S, Hirayama A, Nakamura N, Kimura K, Fujii K, Ishihara M, Saito Y, Tomoike H, Kitamura S. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. Lancet. 2007;370:1483–93.
25. Mitaka C, Kudo T, Jibiki M, Sugano N, Inoue Y, Makita K, Imai T. Effects of human atrial natriuretic peptide on renal function in patients undergoing abdominal aortic aneurysm repair. Crit Care Med. 2008;36:745–51.
26. Bilzer M, Jaeschke H, Vollmar AM, Paumgartner G, Gerbes AL. Prevention of Kupffer cell-induced oxidant injury in rat liver by atrial natriuretic peptide. Am J Physiol. 1999;276:G1137–44.
27. Kiemer AK, Vollmar AM, Bilzer M, Gerwig T, Gerbes AL. Atrial natriuretic peptide reduces expression of TNF-alpha mRNA during reperfusion of the rat liver upon decreased activation of NF-kappaB and AP-1. J Hepatol. 2000;33:236–46.
28. Kiemer AK, Hartung T, Vollmar AM. cGMP-mediated inhibition of TNF-alpha production by the atrial natriuretic peptide in murine macrophages. J Immunol. 2000;165:175–81.
29. Kiemer AK, Gerbes AL, Bilzer M, Vollmar AM. The atrial natriuretic peptide and cGMP: novel activators of the heat shock response in rat livers. Hepatology. 2002;35:88–94.
30. Ishigaki N, Yamamoto N, Jin H, Uchida K, Terai S, Sakaida I. Continuous intravenous infusion of atrial natriuretic peptide (ANP) prevented liver fibrosis in rat. Biochem Biophys Res Commun. 2009;378:354–9.