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Soluble Thrombomodulin Ameliorates Ischemia-Reperfusion Injury of Liver Grafts by Modulating the Proinflammatory Role of High-Mobility Group Box 1

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Transplantation using grafts obtained after cardiac death (CD) is considered a promising solution for graft shortages. However, no standard criteria for organ preservation have been established for CD donors. High-mobility group box 1 (HMGB1) is a DNA-binding protein that is released from dying hepatocytes as an early mediator of inflammation and organ tissue damage. HMGB1 stimulates immunocytes to produce inflammatory cytokines, thereby amplifying the inflammatory response. Thrombomodulin is an integral membrane protein that functions as an endothelial anticoagulant cofactor, and it binds HMGB1 through the extracellular domain. We investigated the effects of ART-123, recombinant human soluble thrombomodulin, on warm ischemia-reperfusion injury in liver grafts. Male Wistar rats were divided into four *ex vivo* groups: heart-beating (HB) group, in which livers were isolated from HB donors; CD group, in which livers were isolated from CD donors exposed to apnea-induced conditions and warm ischemic conditions for 30 min after cardiac arrest; and two CD groups pretreated with ART-123 (1 or 5 mg/kg). Each isolated liver was reperfused for 1 h after cold preservation for 6 h. The perfusate levels of HMGB1, LDH, TNF-*α*, and IL-6 were significantly lower in the CD group pretreated with ART-123 (5 mg/kg) than in the CD group. Bile production was significantly higher in the CD group pretreated with ART-123 (5 mg/kg) than in the CD group. The sinusoidal spaces were significantly narrower in the CD group than in the other groups. We propose that ART-123 maintains sinusoidal microcirculation by reducing endothelial cell damage during warm ischemia-reperfusion injury.

Keywords: ART-123; donation after cardiac death; HMGB1; liver; warm ischemia-reperfusion injury
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Introduction

Liver transplantation is the standard therapy for treating end-stage liver diseases. However, the shortage of available liver grafts has become a global issue. Transplantation using grafts from donation after cardiac death (CD) donors is considered a promising solution to this problem. Recently, CD donors have increased to 10-20% of the liver donor pool in some European countries (Monbaliu et al. 2012). Some researchers have reported that these liver grafts are associated with primary graft nonfunction and biliary complications (Otero et al. 2003). Importantly, no standard criteria or retrieval procedures have been established for CD donors. The fundamental problem with CD organs is prolonged warm ischemia. Organ preservation and transplantation is associated with ischemia-reperfusion (IR) injury (Reddy et al. 2004). Warm ischemia depletes oxygen in cells, thereby reducing intracellular adenosine triphosphate (ATP) levels. ATP depletion leads to pump disarmament, causing the loss of electrolyte gradients and membrane integrity, which in turn promotes cellular edema and sinusoidal narrowing (Carini et al. 1999; Reddy et al. 2004). Thus, warm ischemia causes sinusoidal microcirculatory disturbances after reperfusion (Reddy et al. 2004). IR injury is also associated with liver grafts obtained from CD donors. IR injury involves the release of reactive oxygen species and inflammatory cytokines by Kupffer cells, as well as the activation of neutrophils and the complement system, resulting in cellular damage and injury (Jaeschke 2003; Bahde and Spiegel 2010; Dogan and Aslan 2011).

High-mobility group box 1 (HMGB1) DNA-binding protein is a nuclear factor that is released from cells undergoing necrotic death as a late mediator of lethality in sepsis

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(Wang et al. 1999; Scaffidi et al. 2002). Tsung et al. (2005) reported that in contrast to the delayed role of HMGB1 in sepsis, HMGB1 is released as an early mediator of inflammation and organ damage in hepatic IR injury. Findings from another study suggested that HMGB1 could serve as a marker of hepatocellular injury in human liver transplantation (Ilmakunnas et al. 2008). HMGB1 is released from not only necrotic hepatocytes but also dendritic cell and the Kupffer cell (Ilmakunnas et al. 2008; Ge et al. 2011). HMGB1 induces severe cell damage and propagates inflammatory responses in neighboring cells as it interacts with receptors, including receptor for advanced glycation end-products and the family of Toll-like receptors, such as TLR4 and TLR2 (Wang et al. 1999; Scaffidi et al. 2002; Liu et al. 2011). HMGB1 acts as a chemoattractant for fibroblasts and endothelial and smooth muscle cells, which significantly contributes to wound repair (Ge et al. 2011). Excess HMGB1 expression stimulates immunocytes to produce inflammatory cytokines, thereby amplifying the inflammatory response, tissue factor expression, exacerbation of microcirculatory disturbances, further cell necrosis is caused (Scaffidi et al. 2002; Ilmakunnas et al. 2008). HMGB1 is highly conserved through evolution, and has 99% identity among all mammals. Indeed, out of its 215 amino acids, only two residues are different between the rodent and human versions (Erlandsson Harris and Andersson 2004).

Thrombomodulin is an endothelial anticoagulant cofactor that plays an important role in regulating intravascular coagulation (Esmon 2005). Thrombomodulin is a membrane-bound glycoprotein on vascular endothelial cells (Salem et al. 1984). Thrombomodulin has five domains, an N-terminal lectin-like domain (D1), a domain composed of six epidermal growth factor-like repeats (D2), an O-glycosylation-rich domain (D3), a transmembrane domain (D4), and a cytoplasmic domain (D5) (Suzuki et al. 1987). Thrombin bound to thrombomodulin loses its procoagulating activities, including fibrin formation, platelet aggregation, coagulation factor activation, and activates protein C (Esmon et al. 1982a; Esmon et al. 1982b; Esmon et al. 1983; Esmon 1989; Fuentes-Prior et al. 2000). Recombinant human soluble thrombomodulin (ART-123; Asahi Kasei Pharma Corporation, Tokyo, Japan) has excellent anticoagulant activity and is used to treat disseminated intravascular coagulation (DIC) (Saito et al. 2007). A previous study demonstrated the superiority of ART-123 over low-dose heparin for the treatment of DIC (Saito et al. 2007). Furthermore, some groups have reported that ART-123 has anti-inflammatory effects (Hasegawa et al. 1996; Uchiba et al. 1996; Saito et al. 2007). Previous studies reported that thrombomodulin binds to HMGB1 and is capable of preventing the amplification of inflammatory responses (Abeyama et al. 2005; Ito et al. 2008). Nagato et al. (2009) reported that ART-123 binds to HMGB1 from a necrotic cell and decreases plasma HMGB1 levels in rats.

Miyagi et al. (2004) suggested that warm IR injury causes a feedback loop involving microcirculatory disturbances and cytokine storms, indicating that warm IR injury is similar to DIC. Thus, the anti-inflammatory effects of ART-123 may be beneficial in preventing warm IR injury in liver grafts from CD donors. We previously reported that ART-123 potentially improves the viability of liver grafts from CD donors (Kashiwadate et al. 2012). However, we were unable to clarify the mechanism whereby ART-123 affects warm IR injury in liver grafts.

HMGB1 is an early mediator of inflammation on hepatic IR injury. Thus, we tested the hypothesis that modulating the proinflammatory role of HMGB1 may prevent warm IR injury of liver grafts by using ART-123 in a rat ex vivo model.

Materials and Methods

Animal experiments

Animal experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals," which was prepared by the National Academy of Sciences (National Research Council 2011). The Institutional Animal Care and Use Committee of Tohoku University approved the study (Approval Number: 2012-66).

Experimental design

We constructed an *ex vivo* experimental model of liver perfusion as reported previously (Kashiwadate et al. 2012). All experiments were performed using specific pathogen-free male Wistar rats (Japan SLC, Inc., Shizuoka, Japan), weighing 290-350 g. All rats were housed in a facility designed to maintain appropriate environmental conditions (22-25°C, 12 h light/dark cycle) with access to food and water *ad libitum*. The rats were divided into four *ex vivo* groups, each containing 5 rats: the heart-beating (HB) group, in which livers were isolated while the hearts were beating; the CD group, in which livers were isolated after the rats had been subjected to apnea-induced agonal conditions and remained in warm ischemic conditions for 30 min after cardiac arrest; and the two CD groups pretreated with ART-123 (1 or 5 mg/kg), in which livers were isolated in the same manner as the CD group, but had been pretreated with ART-123 during the agonal stage. The pretreated CD groups were termed 1 mg ART (1 mg/kg ART-123) and 5 mg ART (5 mg/kg ART-123). The dose of 1 mg/kg ART-123 was selected based on previous studies (Mohri et al. 1994; Nagato et al. 2009), and 5 mg/kg ART-123 was used for the high-dose group. All livers were preserved in University of Wisconsin (UW) solution (DuPont Pharmaceuticals Co., Wilmington, DE, USA) for 6 h at 4°C.

Preparation of HB and CD Models

Rats were anesthetized by intraperitoneal pentobarbital administration (50 mg/kg), after which they were administered 1,000 U/kg heparin at the time of laparotomy. The common bile ducts were cannulated with 22-gauge plastic catheters. In the HB group, the portal veins were cannulated with 14-gauge plastic catheters. The livers were flushed *in situ* through the portal veins with cold Ringer's lactate solution (20 mL), and they were subsequently rinsed with cold UW solution (20 mL). The livers were isolated immediately after flushing and preserved at 4°C in UW solution for 6 h. In the CD groups, livers were exposed to apnea-induced agonal conditions caused by an incision made in the diaphragm. Their hearts stopped

beating approximately 10 min after thoracotomy. Their livers were subjected to warm ischemia for 30 min after cardiac arrest, after which they were retrieved and preserved in the same manner as in the HB group. In the 1 mg ART and 5 mg ART groups, the livers were pretreated with a single intravenous dose of ART-123 during the agonal stage. ART-123 was administered as a bolus injection into the tail vein. The volume of ART-123 was less than 1 ml. The control CD group received the vehicle treatment, in which an equal volume of physiologic saline was administered.

Perfusion

We used previously described conditions for the liver perfusion (Itasaka et al. 1999; Dutkowski et al. 2006; Ferrigno et al. 2011; Kashiwadate et al. 2012). The perfusion circuit was composed of a non-recirculating Perfusion System PS-1 (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) filled with Krebs-Henseleit bicarbonate buffer (pH 7.4) at 37°C. A gas mixture comprising 95% oxygen and 5% carbon dioxide was bubbled through the buffer at a partial oxygen pressure of 450-500 mm Hg. After cold preservation, livers from all groups were connected to the perfusion circuit through the portal vein. Subsequently, the livers were perfused at constant pressure for 1 h with Krebs–Henseleit bicarbonate buffer in the PS-1 system. At the time of perfusion, the inflow pressure was measured in real time, using a Research Grade Blood Pressure Transducer (110 VAG/60 Hz; Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). The livers were perfused at a constant pressure of 7 mm Hg. Liver specimens were obtained 1 h after perfusion, freeze-clamped in liquid nitrogen, and stored at −80°C until analysis.

HMGB1 levels

HMGB1 levels in the perfusate were measured 1 h after perfusion using the HMGB1 ELISA Kit II (Shino-Test Corporation, Tokyo, Japan).

Bile production and portal flow volume

Bile production was measured for 1 h to examine liver function (Nakano et al. 1997; Taneja et al. 1998; Miyagi et al. 2004). Bile was collected through the cannulated common bile duct. Perfusates were collected to measure portal flow volume for 1 h.

Liver function

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), levels in the perfusate were measured 1 h after perfusion using the TA-LN Kainos kit (Kainos Laboratories Inc., Tokyo, Japan), whereas lactate dehydrogenase (LDH) levels were measured using the Espa LDH liquid kit (Nipro Corporation, Osaka, Japan).

Cytokines

Tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 levels in the perfusate were measured 1 h after perfusion using commercially available enzyme-linked immunosorbent assay kits (TNF alpha ELISA Kit, Rat; IL-1 beta ELISA Kit, Rat; and IL-6 Rat ELISA Kit; Invitrogen Corporation, Camarillo, CA, USA).

Histological examination

Ten tissue sections from each of the livers were stained with hematoxylin and eosin and examined under an optical microscope. Histological evaluation was performed using the morphological classification of hepatic injury in the isolated perfused rat liver (IPRL) model (Tojimbara et al. 1997; Martin et al. 2000; Bessems et al. 2006), graded on a scale of 1 (excellent) to 9 (poor): (1) normal rectangular structure of the hepatocytes, (2) rounded hepatocytes with an increase of sinusoidal spaces, (3) vacuolization in zone 3; (4) vacuolization in zone 2; (5) vacuolization in zone 1; (6) vacuolization and nuclear pyknosis in zone 3; (7) vacuolization and nuclear pyknosis in zone 2; (8) vacuolization and nuclear pyknosis in zone 1 and (9) necrosis. The sinusoidal space was measured using the open-source image processing software, Image J version 1.44 (National Institutes of Health, Bethesda, MD, USA) (Kashiwadate et al. 2012).

Statistical analyses

All statistical analyses were performed using the JMP Pro 11 software package (SAS Institute Inc., Cary, NC, USA). All values are presented as the mean ± standard deviations. Statistical analyses were conducted using 1-way analysis of variance and the Dunnet test, in which a control group is the CD group. $P < 0.05$ was considered statistically significant.

Results

HMGB1 levels

The mean HMGB1 level in the perfusate was significantly lower in the HB and 5 mg ART groups than in the CD group (Fig. 1).

Bile production and portal flow volumes

Bile production, which reflects liver function (Nakano et al. 1997; Taneja et al. 1998), was significantly higher in the HB and 5 mg ART groups than in the CD group (Fig. 2). Portal flow volumes in the HB, 1 mg ART, and 5 mg ART groups were significantly higher than in the CD group (Table 1).

Liver function

AST and ALT levels were significantly lower in the HB group than in the CD group. The mean LDH level was significantly lower in the HB and 5 mg ART groups than in the CD group (Table 1).

Cytokine levels

Inflammatory cytokine levels in perfusates were measured to evaluate the anti-inflammatory effect of ART-123. These inflammatory cytokines were released mainly from Kupffer cell. The mean TNF-α level was significantly lower in the 5 mg ART group than in the CD group (Fig. 3A). The mean IL-1β level was significantly lower in the HB group than in the CD group (Table 1). The mean IL-6 level was significantly lower in the HB, 1 mg ART, and 5 mg ART groups than in the CD group (Fig. 3B).

Histological results

Histological evaluation was performed using the morphological classification of hepatic injury (Fig. 4). Sinusoid structures were well preserved in the HB group, which was classified as score 2 (rounded hepatocytes with an increase of sinusoidal spaces). The CD group was classified as score

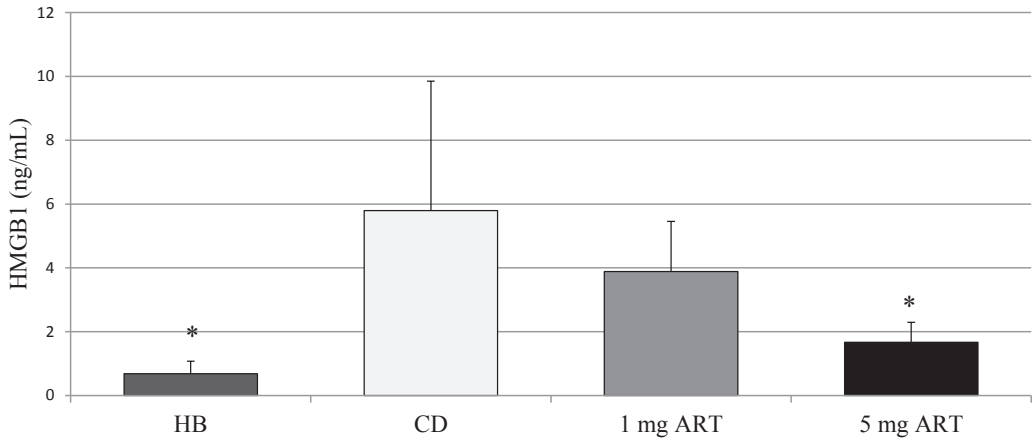

Fig. 1. Perfusate concentrations of HMGB1.
The mean perfusate level of high mobility group box 1 (HMGB1 was significantly lower in the HB and 5 mg ART groups than in the CD group. HB, heart-beating group; CD, cardiac death group; 1 mg ART, CD group pretreated with 1 mg/kg ART-123; 5 mg ART, CD group pretreated with 5 mg/kg ART-123. All values are presented as the mean ± standard deviation (n = 5). *P < 0.05, compared to the CD group.

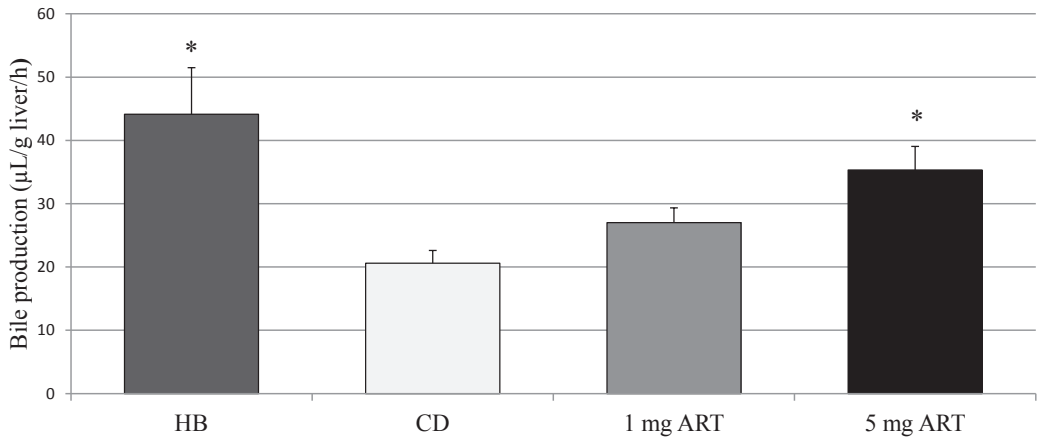

Fig. 2. Bile production during reperfusion.
Bile production was measured for 1 h. Bile production in the HB and 5 mg ART groups was significantly higher than in the CD group. HB, heart-beating group; CD, cardiac death group; 1 mg ART, CD group pretreated with 1 mg/kg ART-123; 5 mg ART, CD group pretreated with 5 mg/kg ART-123. All values are presented as the mean ± standard deviation (n = 5). *P < 0.05, compared to the CD group.

5 (hepatocellular vacuolization in zones 1, 2, and 3). Hepatocellular vacuolization reflects the severity of hepatocellular damage (Monbaliu et al. 2008). The 1 mg ART and 5 mg ART groups were classified as score 3 (hepatocellular vacuolization in zone 3), but the structure of the sinusoid was well preserved in those groups. In the CD group, the sinusoidal space was narrowed because the hepatocytes were enlarged. The sinusoidal space was significantly narrower in the CD group than in the other groups (Table 1).

Discussion

HMGB1 is an early mediator of inflammation and organ damage following hepatic IR injury (Tsung et al. 2005). In this study, HMGB1 levels in perfusates of the CD group were significantly higher than those in the HB group. We found that ART-123 administration in the CD group during the agonal stage decreased perfusate HMGB1 levels. In the 5 mg ART group, the perfusate HMGB1 level was significantly lower than that in the CD group. In addition, in the 5 mg ART group, bile production and portal flow volumes increased, the extent of hepatocellular vacuolation decreased, the sinusoidal space was maintained, LDH expression was inhibited, and TNF-α and IL-6 production decreased. In the 1 mg ART group, perfusate HMGB1 levels were slightly lower than those in the CD group, portal flow volume increased, IL-6 production decreased, the extent of hepatocellular vacuolation decreased, and the sinusoidal space was maintained. ART-123 binds HMGB1, thereby decreasing TNF-α production, protecting sinusoidal endothelial cells, and preserving the microcirculation. These effects are thought to improve the function of CD livers.

Table 1. Portal flow volume, AST, ALT, LDH, IL-1β, and sinusoidal space measured 1 h after reperfusion.

Group	HB	CD	1 mg ART	5 mg ART
Portal flow volume (mL/g liver/h)	241.5 ± 15.6*	183.9 ± 16.6	232.3 ± 19.5*	248.4 ± 30.1*
AST (Karmen units)	2.8 ± 0.3*	5.2 ± 1.6	4.0 ± 1.0	3.6 ± 0.5
ALT (Karmen units)	2.4 ± 0.4*	4.7 ± 1.4	3.6 ± 1.1	3.1 ± 0.6
LDH (IU/L)	35.4 ± 8.2*	73.3 ± 12.1	89.8 ± 23.4	37.9 ± 7.6*
IL-1β (pg/mL)	10.8 ± 1.6*	17.5 ± 3.6	14.5 ± 3.9	13.2 ± 3.3
Sinusoidal space (%)	16.4 ± 1.4*	12.2 ± 1.8	17.7 ± 0.6*	20.4 ± 0.9*

HB, heart-beating group; CD, cardiac death group; 1 mg ART, CD group pretreated with 1 mg/kg ART-123; 5 mg ART, CD group pretreated with 5 mg/kg ART-123; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; IL-1β, interleukin-1β. Data are presented as the means ± standard deviations (n = 5). *$P < 0.05$ compared to the CD group.

HMGB1 is a late mediator of lethality in sepsis (Wang et al. 1999; Scaffidi et al. 2002). In contrast, HMGB1 is released as an early mediator of inflammation and organ damage in hepatic IR injury (Tsung et al. 2005). Inhibition of HMGB1 decreases the damage in hepatic IR injury (Tsung et al. 2005; Watanabe et al. 2005; Izuishi et al. 2006). Additionally, binding of ART-123 to HMGB1 allows for the inhibition of the amplification of inflammatory response (Abeyama et al. 2005; Ito et al. 2008). We tested the hypothesis that control of HMGB1 using ART-123 prevents warm IR injury of liver grafts. Furthermore, we used a rat model to evaluate only the anti-inflammatory effects of ART-123 (Mohri et al. 1997). This is the first report to reveal that liver graft viability is potentially improved by inhibition of HMGB1 using ART-123 during warm IR injury in liver grafts.

Some reports have demonstrated an anti-inflammatory activity of thrombomodulin (Hasegawa et al. 1996; Uchiba et al. 1996). One mechanism underlying the anti-inflammatory activity of thrombomodulin involves the binding of thrombin and thrombomodulin. This thrombin-thrombomodulin complex activates protein C. Activated protein C (APC) retains binding affinity for the endothelial cell protein C receptor (EPCR). Once APC dissociates from the EPCR, it binds to protein S and proteolytically inactivates factors Va and VIIIa (Esmon 2005). APC has been shown to have anti-inflammatory, cytoprotective, and anti-apoptotic effects via EPCR and protease-activated receptor-1 (O'Brien et al. 2007). Recent findings have shown that the D1 of thrombomodulin has anti-inflammatory properties and binds to HMGB1 (Abeyama et al. 2005; Ito et al. 2008). ART-123 is a recombinant form of human soluble thrombomodulin that activates protein C in both primates and humans (Mohri et al. 1997). In rats, ART-123 does not activate protein C (Mohri et al. 1997), and its anticoagulant activity is weak. Therefore, our model was less affected by anticoagulant therapy, and thus this model was appropriate for evaluating the effects of inhibiting HMGB1 activity with ART-123. In this study, the D1 of ART-123 may bind to HMGB1, which decreases HMGB1 levels to that of the HB group, thereby decreasing TNF-α production, protecting sinusoidal endothelial cells, and preserving the microcirculation. The D1 of ART-123 may bind to the excess HMGB1 released from necrotic hepatocytes, and stops a vicious circle on warm IR injury of liver grafts.

The livers from uncontrolled CD donors were negatively affected, not only by warm ischemia, but also by the release of TNF-α and endotoxin following intestinal ischemia (Zhang et al. 2000). In our CD model, the livers underwent an apnea-induced agonal condition. Agonal condition was defined as systemic blood flow gradually deteriorated. Cardiac arrest was induced approximately 10 min after thoracotomy. Zhang et al. (2000) reported that, after cardiac arrest, mitochondrial proton ATPase activity, TNF-α levels, and partial oxygen pressure differed significantly between CD-model animals induced by potassium chloride versus those induced by thoracotomy. Thus, we suggest that our model is suitable for investigating CD donors. We administered heparin prior to performing graft extractions, which is a typical procedure during clinical

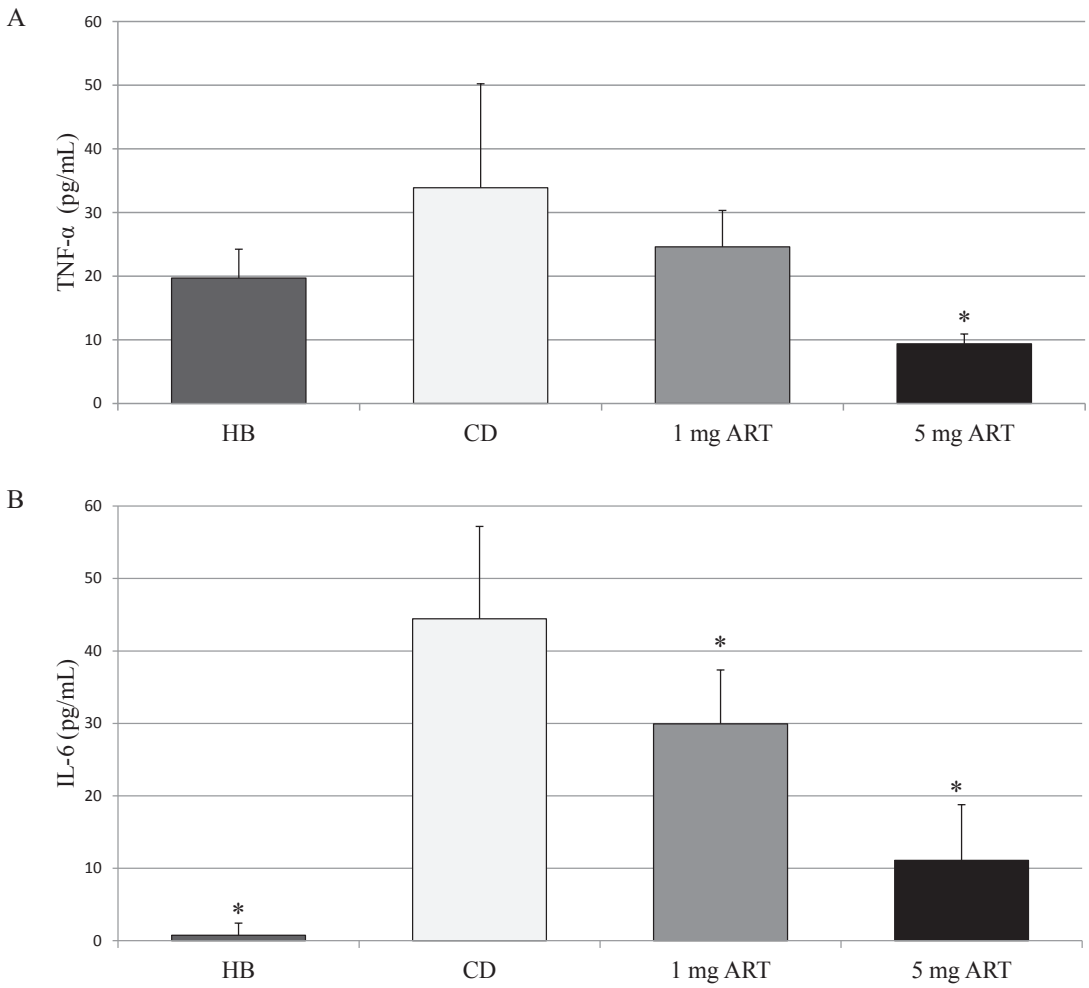

Fig. 3. Concentrations of inflammatory cytokines in perfusates.
(A) The mean TNF-α level was significantly lower in the 5 mg ART group than in the CD group. (B) The mean IL-6 level was significantly lower in the HB, 1 mg ART, and 5 mg ART groups than in the CD group. HB, heart-beating group; CD, cardiac death group; 1 mg ART, CD group pretreated with 1 mg/kg ART-123; 5 mg ART, CD group pretreated with 5 mg/kg ART-123. All values are presented as the mean ± standard deviation (n = 5). *P < 0.05, compared to the CD group.

donor operations. Previous studies have reported that liver grafts remain suitable for transplantation purposes for 30–60 min after warm ischemia in the absence of agonal conditions (Takada et al. 1997; Monbaliu et al. 2005). However, another study reported that no liver-transplant recipients survived for 24 h in cases where the grafts underwent 30 min of warm ischemia with agonal conditions (Miyagi et al. 2009). Considering these past findings, we set the warm ischemia time to 30 min and used an IPRL model, which has been widely used to evaluate graft viability after liver transplantation (Bessems et al. 2006).

This study has some limitations. There are ethical concerns regarding treating donors at the agonal stage prior to cardiac arrest. However, to confirm the inhibitory role of ART-123 for HMGB1, we administered it immediately prior to cardiac arrest, when the effect would be notable. In addition, ART-123 is an approved drug for treatment of DIC and can be considered for clinical applications. Our findings indicate that ART-123 has cytoprotective effects on liver grafts and reduces endothelial cell damage. However, the evaluation of endothelial cells was only by HE staining. In future studies, we should perform transmission electron microscopy and silver staining.

The clinical dose used for the treatment of DIC is 380 U/kg (0.06 mg/kg), but higher doses of ART-123 were necessary in this study to detect its protective effect on warm IR injury. Thus, it is difficult to extrapolate the findings from this study to clinical applications. The data of this ex vivo experiment do not necessarily reflect the clinical condition. However, the results of this study may enhance the understanding of the early phase of warm IR injury and the related anti-inflammatory effects of HMGB1.

In conclusion, our findings suggest that ART-123 may inhibit the proinflammatory function of HMGB1, which ameliorates endothelial cell damage and sinusoidal microcirculation disorders during warm IR injury. ART-123 may improve liver graft viability, making it a promising strategy for liver transplantation from CD donors.

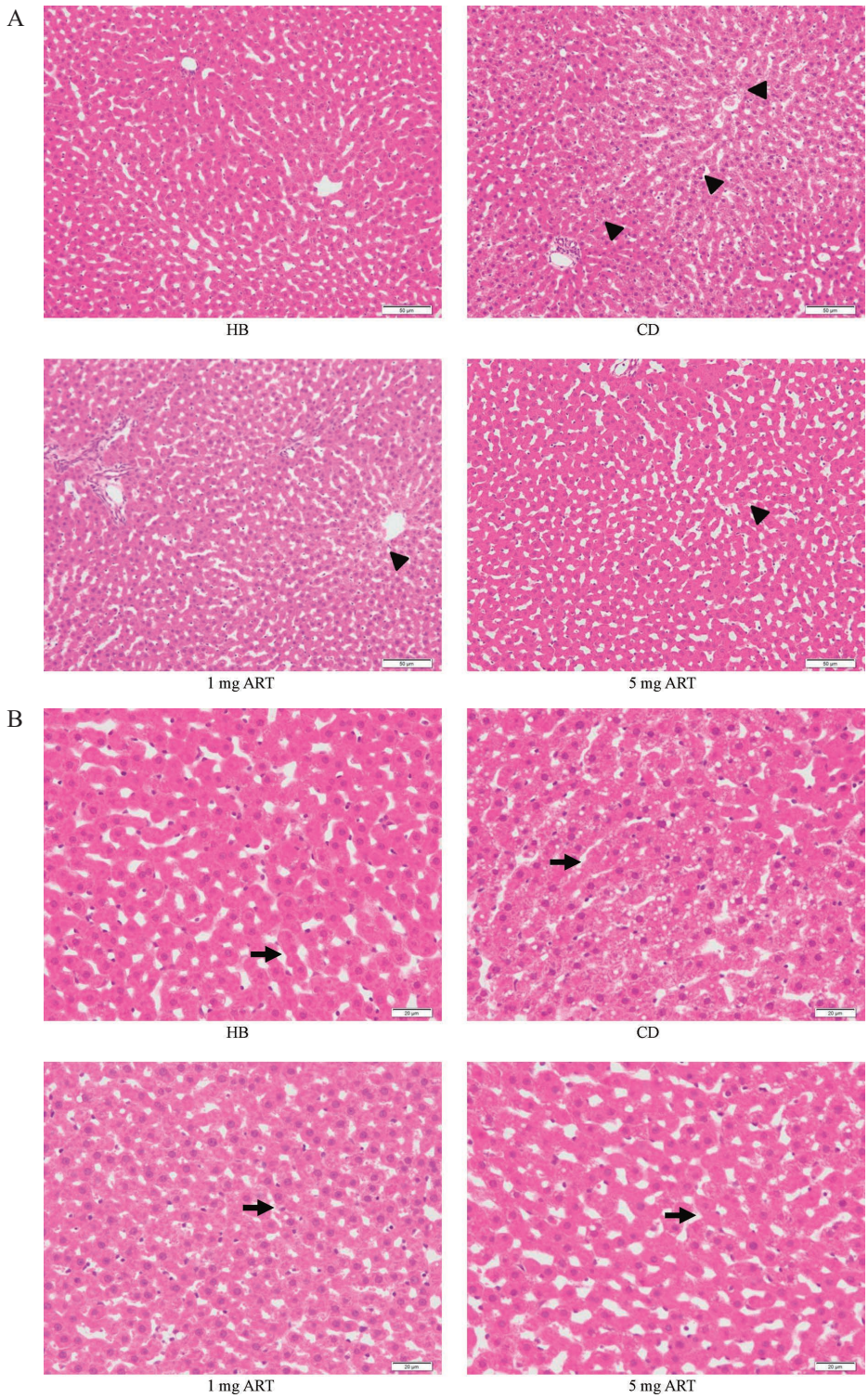

Fig. 4. Histological findings.
HB group, CD group, 1 mg ART group, and 5 mg ART group. Sinusoid structures were well preserved in the HB, 1 mg ART, and 5 mg ART groups. In contrast, the sinusoidal space was relatively narrow in the CD group, in which the hepatocytes were enlarged. The HB group was classified as score 2 (rounded hepatocytes with an increase of sinusoidal spaces). The CD group was classified as score 5 (hepatocellular vacuolization (arrowheads) in zones 1, 2, and 3). The 1 mg ART and 5 mg ART groups were classified as score 3 (hepatocellular vacuolization (arrowheads) in zone 3). Scale bar = 50 μm. (B) The enlarged image of each tissue section. The sinusoidal space (arrows) was relatively narrow in the CD group. Scale bar = 20μm.
HB, heart-beating group; CD, cardiac death group; 1 mg ART, CD group pretreated with 1 mg/kg ART-123; 5 mg ART, CD group pretreated with 5 mg/kg ART-123.

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Conflict of Interest

The authors declare no conflict of interest.

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