Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.14004

HEPATOLOGY

Phosphodiesterase III inhibitor attenuates rat sinusoidal obstruction syndrome through inhibition of platelet aggregation in Disse's space

Takashi Miyata,* Hidehiro Tajima,* Miki Hirata,* Shin-ichi Nakanuma,* Isamu Makino,* Hironori Hayashi,* Katsunobu Oyama,* Tomoharu Miyashita,* Hiroyuki Takamura,* Itasu Ninomiya,* Sachio Fushida,* Shoichi Iseki,† Shin-ichi Harada,‡ Tomohiko Wakayama§ and Tetsuo Ohta*

*Department of Gastroenterological Surgery, Division of Cancer Medicine, †Department of Histology and Embryology, Division of Cancer Medicine, ‡Center for Biomedical Research, Graduate School of Medicine, Kanazawa University, Kanazawa, Ishikawa, and §Department of Histology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Key words
cilostazol, extravasated platelet aggregation, plasminogen activator inhibitor-1, platelets, sinusoidal obstruction syndrome.

Accepted for publication 13 September 2017.

Correspondence
Hidehiro Tajima, Department of Gastroenterological Surgery, Division of Cancer Medicine, Graduate School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.
Email: hidetaji@staff.kanazawa-u.ac.jp

Declaration of conflict of interest: We have no financial conflicts of interest to disclose concerning the study.

Abstract

Background and Aim: Sinusoidal obstruction syndrome (SOS) is a serious drug-induced liver injury. However, the pathophysiology of the disease remains unclear. This study investigated the effects of cilostazol (CZ), a phosphodiesterase III inhibitor, in a monocrotaline (MCT)-induced rat model of SOS.

Methods: Male Wistar rats were administrated MCT to induce SOS. Rats were divided into control, MCT, and MCT + CZ groups. In the MCT + CZ group, CZ was administered at 48 h, 24 h, and 30 min prior to and 8 h and 24 h after MCT administration. The MCT group was treated with water instead of CZ. At 48 h after MCT administration, blood and liver samples were collected to assess biochemistry and liver histology. Expression of rat endothelial cell antigen, CD34, CD41, P-selectin, and caspase-3 in the liver were analyzed. Plasminogen activator inhibitor-1 (PAI-1) in hepatocytes was analyzed using western blotting and polymerase chain reaction.

Results: In the MCT group, macroscopic findings showed a dark-red liver surface. Histological findings showed sinusoidal dilatation, coagulative necrosis of hepatocytes, and endothelial damage of the central vein. These changes were attenuated in the MCT + CZ group. Elevated serum transaminase and decreased platelet counts were observed in the MCT + CZ group compared with those in the MCT group. Treatment with CZ reduced MCT-induced damage to the liver sinusoidal endothelial cells, inhibited extravasated platelet aggregation, and suppressed hepatocyte apoptosis around the central vein. CZ attenuated hepatic PAI-1 protein and mRNA levels.

Conclusions: Cilostazol attenuated MCT-induced SOS by preventing damage to liver sinusoidal endothelial cells and extravasated platelet aggregation. Hepatic PAI-1 levels were suppressed with CZ treatment.

Introduction

Sinusoidal obstruction syndrome (SOS), previously called veno-occlusive disease,[1] is a fatal drug-induced liver injury. Drugs such as busulfan in hematopoietic stem cell transplantation,[2,3] cyclophosphamide in immunosuppression therapy and bone marrow transplantation,[4] and oxaliplatin in chemotherapy[5,6] are known to cause SOS. The clinical features of SOS include hyperbilirubinemia, painful hepatomegaly, and weight gain due to ascites.[2,7] These features decrease the hepatic functional reserve.[8] Prevention and treatment of SOS are necessary to improve complications following liver surgery; however, an effective strategy for SOS remains to be determined.

Several studies have reported that initial pathophysiological changes in SOS are possibly the result of drug-induced injury to liver sinusoidal endothelial cells (LSEC),[4,9–16] but mechanisms after LSEC damage remain unclear. We previously immunostained for CD42b, a platelet surface marker, and observed platelets in contact with hepatocytes, especially in zone 3, in the liver tissue of a liver transplant recipient with severe SOS. It was suggested that platelets exist in the extravascular space, the space of Disse, and destruction of hepatocytes were observed.[17,18] Because of a lack of nuclei, platelets are invisible on histological analysis using hematoxylin and eosin (HE) staining.

We considered that LSEC are injured in the liver of SOS patients, and platelets that aggregate in the extravascular space, the space of Disse, play an important role in processes resulting in SOS. We termed platelet aggregation in the extravascular space as extravasated platelet aggregation (EPA).[17–19] In the current study, we hypothesized that to prevent LSEC damage and instead of antiplatelet therapy, anti-EPA therapy is beneficial for SOS and results in the promotion of liver regeneration. To this

end, we used a phosphodiesterase (PDE) III inhibitor, which has a protective effect on LSEC[9] and inhibits platelet aggregation, to investigate the pathophysiology of SOS and the efficacy of PDE-III inhibitor in the pharmacological prevention of SOS in the liver of a rat model of SOS.

Methods

Reagents. Monocrotaline (MCT) is a pyrrolizidine alkaloid found in *Crotalaria* and was purchased from Wako Pure Chemical Industries (Osaka, Japan). To prepare a solution of MCT at 20 mg/mL, 1000 mg of MCT was dissolved in 1.0 N HCl, and the pH was adjusted to 7.4 with 0.5 N NaOH. Phosphate-buffered saline (pH 7.4) was added to increase the total volume to 50 mL.[5,20] Cilostazol (CZ), a PDE-III inhibitor, was provided by Otsuka Pharmaceutical Co. (Tokyo, Japan) and dissolved in water with carboxymethyl cellulose sodium salt (Wako Pure Chemicals). CZ is a specific inhibitor of PDE-III and has been studied for its inhibitory effects on platelet aggregation via increased cyclic adenosine monophosphate levels.

Animals. Male Wistar rats (230–300 g; Charles River Laboratories, Inc., Japan) were used and had free access to water and standard laboratory chow. The study was conducted in compliance with the Division for Animal Research Resources, University of Kanazawa, and experiments and procedures were approved by the Animal Care and Use Committee, University of Kanazawa.

Experimental protocol. Rats were randomly divided into three groups (n = 10 per group): control, MCT, and MCT + CZ. The protocol is shown in Figure 1. Rats had access to water and standard laboratory chow *ad libitum* but were fasted for 12 h before the administration of MCT (90 mg/kg). In the MCT + CZ group, 30 mg/kg CZ was administered orally at 48 h, 24 h, and 30 min prior to and 8 and 24 h after administration of MCT. In the other groups, solvent water was administered orally at the same time periods. Histopathological changes at 48 h after administration of MCT in rats are similar to those in human SOS.[5,21] Rats were anesthetized by inhalation of diethyl ether and killed for the collection of serum from the inferior vena cava and liver tissue.

Macroscopic findings. A midline incision was made. Tissues were examined for the accumulation of peritoneal fluid and the color of the liver surface to determine the effects of CZ.

Biochemical analysis. Blood samples were taken, and white blood cells, hemoglobin, and platelets were counted using an automated blood cell counter (Celltac α MEK-6458; Nihon Kohden, Tokyo, Japan). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), direct bilirubin, lactate dehydrogenase, and hyaluronic acid (HA) were measured in the same samples. Measurements were performed by SRL Inc., Japan.

Histological analysis. Liver tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and cut serially into 4-μm sections. Slides were prepared and stained with HE and

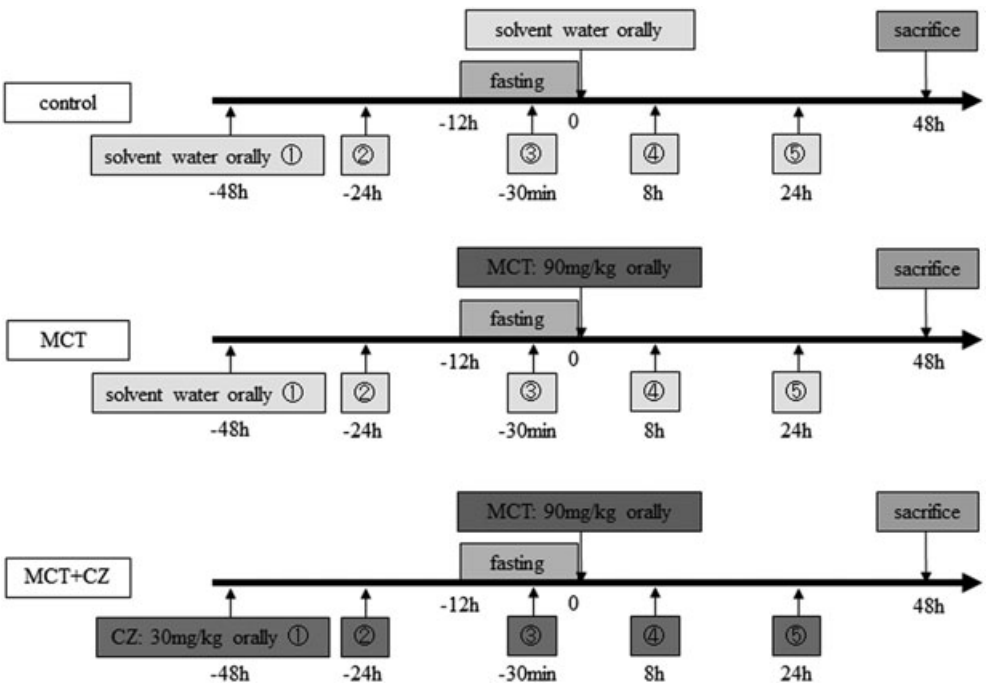

Figure 1 Experimental protocol. Isotonic sodium chloride solution was administered orally instead of MCT in the control group. In all groups, blood and liver samples were collected at 48 h after either MCT or solvent water treatment. CZ, cilostazol; MCT, monocrotaline.

evaluated in 10 randomly. To quantify the degree of SOS, histological changes were determined according to sinusoidal dilatation, coagulative necrosis of hepatocytes, endothelial damage of the central vein, and sinusoidal hemorrhage.[5,21,22] Each of these features was graded on a 4-point scale: 0 = absent; 1 = mild (1–30%); 2 = moderate (31–60%); 3 = severe (61–100%). The total SOS score was calculated as the sum of individual scores.

Immunohistochemistry. For assessment of damage to LSEC, immunostaining for rat endothelial cell antigen 1 (RECA-1) (#MCZ-970R; Serotec, Oxford, UK) and CD34 (1:20, AF4117; R&D Systems, Minneapolis, MN, USA) was performed. Tissue samples were fixed with 4% paraformaldehyde phosphate buffer solution for 3 days and embedded in a solution of optimal cutting temperature compound (Sakura Finetek, Tokyo, Japan), 30% sucrose in phosphate buffer (0.1 M, pH 7.4), and 0.05% NaN_3. Tissue samples were sectioned (6 μm thick) using cryostats (Thermo Fisher Scientific, Waltham, MA, USA). For assessment of platelet aggregation, immunostaining for CD41 antibody (1:100 orb4832; Biorbyt, Cambridge, UK) and P-selectin (1:50, ERP1444(2)(B); Abcam, Tokyo, Japan) was performed. For assessment of liver apoptosis, immunostaining for anti-cleaved caspase-3 antibody (1:100 9661; Cell Signaling Technology, Beverly, MA, USA) was performed.

Reverse transcription quantitative PCR analysis. Total RNA was extracted from liver tissues using an RNeasy mini kit (Qiagen, Tokyo, Japan). Residual DNA in isolated total RNA preparations was removed by treatment with DNase (Qiagen), and 5 μg RNA was reverse-transcribed using SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Primer sets used were for plasminogen activator inhibitor-1 (PAI-1) and for glyceraldehyde phosphate dehydrogenase as an internal control. Primers were synthesized by Hokkaido System Science (Hokkaido, Japan). Real-time polymerase chain reaction (PCR) was performed using Mx QPCR Systems and Complete QPCR Portfolio (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. All cDNA samples were analyzed in triplicate, and each run contained a relative standard curve.

Western blotting. Total protein was extracted from liver tissues kept at −80 °C in T-PER tissue protein extraction reagent (Pierce Biotechnology, Rockford, IL, USA). A ratio of 0.01 g of tissue to 80 μL T-PER reagent and 0.4 μL protease inhibitor was mixed and homogenized. Samples were centrifuged and supernatant collected for analysis. Samples were separated on 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA). PAI-1 antibody (ab7205; Abcam, Cambridge, UK) at 1:1000 dilution and β-actin (Sigma-Aldrich, St. Louis, MI, USA) at 1:10 000 were used as the primary antibodies. The antibody–antigen complex was detected using a light-capture system (Atto, Tokyo, Japan) and a CS analyzer program (Atto).

Statistical analysis. Results are expressed as mean ± standard deviations. Comparisons between two groups were performed using Student's *t*-tests, as appropriate. A P-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 11.0.1 (SPSS Inc., Chicago, IL, USA).

Results

Morphology. At sacrifice, macroscopic liver findings in the MCT group showed bloody ascites and the liver surface appeared congested and dark red. These changes were attenuated in the MCT + CZ group (Fig. 2).

Blood chemistry. The MCT and MCT + CZ groups showed bloody ascites, but blood cell counts showed no significant change in hemoglobin between the groups (Fig. 3). White blood cell and platelet counts significantly decreased in the MCT group compared with those in the control group. These decreases were suppressed in the MCT + CZ group (Fig. 3). The results of serum biochemistry tests are shown in Table 1. Serum AST, ALT, T-Bil, direct bilirubin, and HA in the MCT + CZ group were significantly lower than those in the MCT group ($P = 0.007$, $P = 0.028$, $P = 0.005$, $P = 0.003$, $P = 0.010$, respectively).

Histopathological findings. The HE staining of livers showed sinusoidal dilatation, coagulative necrosis of hepatocytes, endothelial damage of the central vein, and sinusoidal hemorrhage in the MCT group. In the MCT + CZ group, these morphological changes were attenuated (Fig. 4). The results were reflected in HE staining scores for each of these four histological features and the total SOS score. Scores for the MCT + CZ group were significantly lower than those in the MCT group (Table 2).

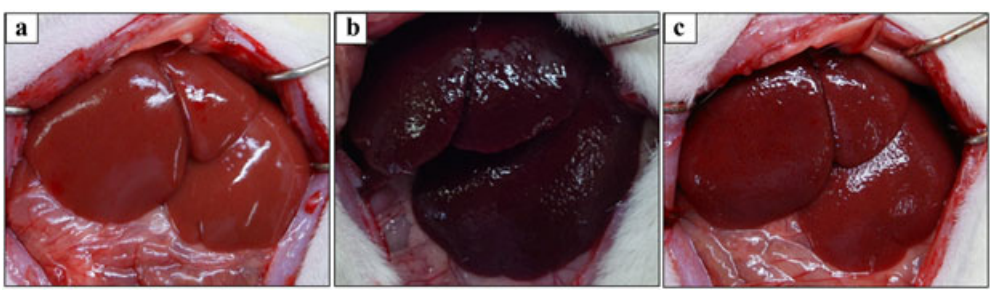

Figure 2 Macroscopic findings. (a) Control, (b) monocrotaline, and (c) monocrotaline + cilostazol groups. (b) Accumulation of bloody ascites and the liver surface appeared dark red. (c) The changes were attenuated.

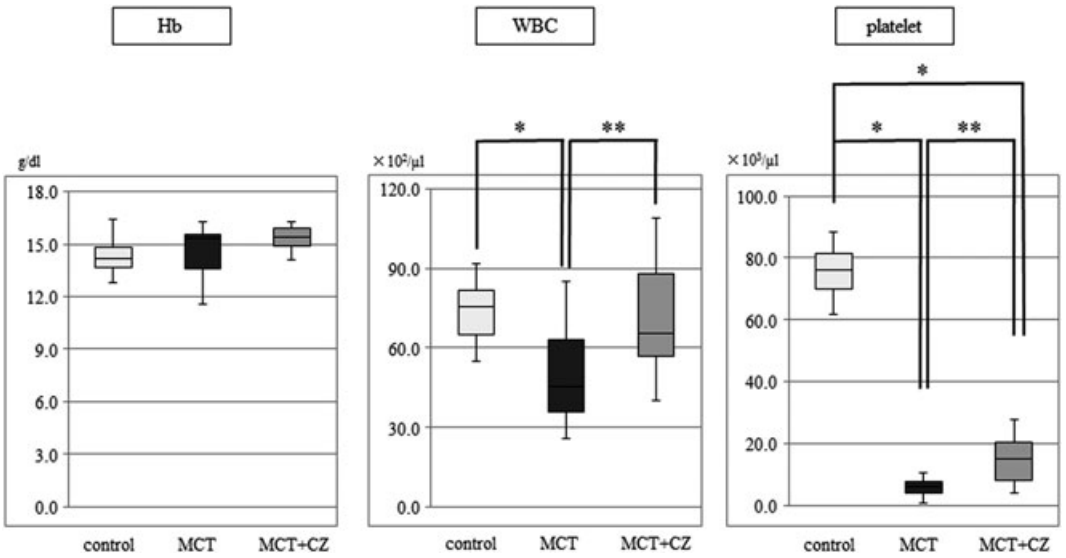

Figure 3 Comparison of hemoglobin (Hb), platelet, and white blood cell (WBC) counts among the three groups. Blood cell counts showed no significant changes in Hb among the three groups. The platelet count significantly decreased in the monocrotaline (MCT) and MCT + cilostazol (CZ) groups. This decrease was suppressed by the administration of CZ. The WBC count decreased significantly in the MCT group. This decrease was suppressed by the administration of CZ. The WBC count was maintained in the MCT + CZ group. Data are expressed as mean ± standard deviations (n = 10). *$P < 0.01$ *versus* control. **$P < 0.01$ *versus* MCT.

Table 1 Serum biochemistry at 48 h after MCT administration

Variable	Normal range	MCT group	MCT + CZ group
AST (IU/L)	71–100	7218 ± 4071	2644 ± 1876*
ALT (IU/L)	30–44	1539 ± 837	760 ± 578*
T-Bil (mg/dL)	0.03–0.05	0.211 ± 0.0836	0.113 ± 0.387*
D-Bil (mg/dL)	0.03–0.04	0.126 ± 0.0538	0.0570 ± 0.0231*
LDH (IU/L)	110–580	5664 ± 3701	3276 ± 2180
HA (ng/dL)	25–60	1011.1 ± 615.7	379.4 ± 144.8*

*$P < 0.01$ compared with MCT.

Although the administration of monocrotaline (MCT) resulted in considerable elevation in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), direct bilirubin (D-Bil), lactate dehydrogenase (LDH), and hyaluronic acid (HA), cilostazol (CZ) resulted in a noticeable decrease in serum AST, ALT, T-Bil, D-Bil, and HA compared with that in the MCT group. Data are mean ± standard deviations (n = 10).

Immunohistochemistry. Rat endothelial cell antigen 1 protein expression in the MCT group was markedly reduced compared with that in the control group. RECA-1 protein expression in the MCT + CZ group was comparable with that in the control group (Fig. 4). There was almost no CD34 expression in the control group, but CD34-positive areas were significantly large in the MCT group. Administration of CZ attenuated CD34 expression (Fig. 4). CD41 protein expression in the control group was absent. In the MCT group, CD41 protein expression was only observed in contact with hepatocytes, especially in zone 3. These changes were attenuated in the MCT + CZ group (Fig. 4). P-selectin protein expression was higher in the MCT group compared with that in the control group. In the control group, there was partial endothelial cell staining. In the MCT group, endothelial cells and platelets around the central vein were stained. In the MCT + CZ group, these changes decreased (Fig. 4). In the control group, cleaved caspase-3-labeled cells were absent. Cells positive for cleaved caspase-3 labeling in the MCT group were observed at a greater extent in the MCT group compared with those in the MCT + CZ group (Fig. 4).

PAI-1 expression. Results from reverse transcription quantitative PCR analysis are shown in Figure 5. PAI-1 mRNA expression in the MCT and MCT + CZ groups were significantly higher than that in the control group (P = 0.003 and P = 0.016, respectively). There was no significant difference between the MCT group and the MCT + CZ group. PAI-1 mRNA in the MCT + CZ group tended to show a reduction compared with that in the MCT group. With western blotting, hepatic PAI-1 protein expression in the MCT group was higher than that in the MCT + CZ group (Fig. 5).

Discussion

Sinusoidal obstruction syndrome is a drug-induced liver injury resulting in multi-organ failure and death[4] and is mostly associated with hematopoietic stem cell transplantation,[2,3] cyclophosphamide in immunosuppression therapy for liver transplants,[4] and chemotherapy such as oxaliplatin.[5,6] It is characterized by hyperbilirubinemia, painful hepatomegaly, and weight gain due to ascites.[2,7] In recent years, increasing numbers of studies have described the mechanisms behind SOS and suggest that the first pathological change appears in the sinus structure.[12,14,23] Reports suggest that exposure to drug toxins result in swelling and accumulation of LSEC, especially in zone 3.[17,24] Subsequent pathological changes in SOS appear as sinus fibrosis, fibroblast cell

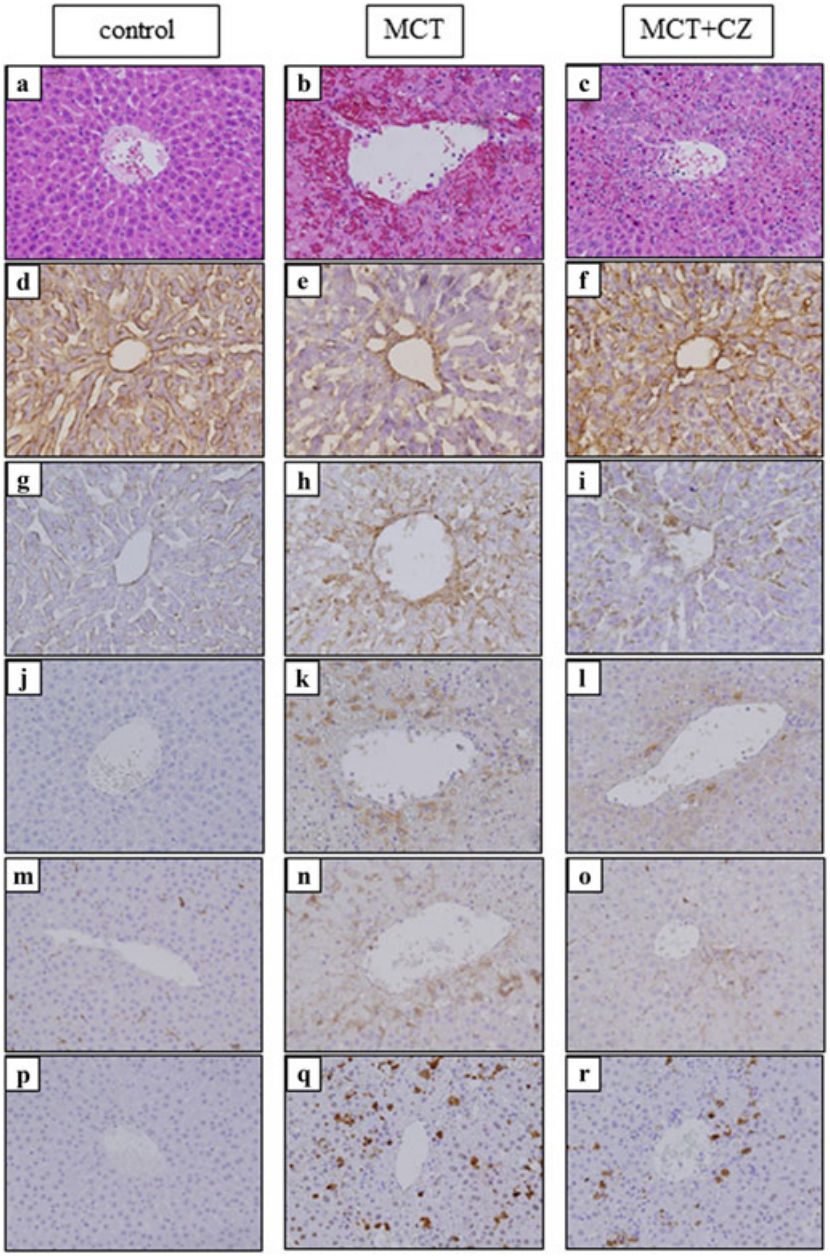

Figure 4 Histological analysis (original magnification ×400). a–c: HE staining. (a) Control, (b) monocrotaline (MCT), and (c) MCT + cilostazol (CZ) groups. In the control group, there was no evidence of sinusoidal dilatation, coagulative necrosis of hepatocytes, endothelial damage of the central vein, and sinusoidal hemorrhage. Administration of MCT induced severe sinusoidal hemorrhage, endothelial damage of the central vein, and coagulative necrosis of hepatocytes. Administration of CZ resulted in improvements to pathological changes in the liver induced by the administration of MCT. d–f: Immunohistochemistry of rat endothelial cell antigen (RECA)-1. (d) Control, (e) MCT, and (f) MCT + CZ groups. Immunohistochemical analysis indicated that the RECA-1-positive protein expression area in the control group was widespread and reflected normal sinusoidal lining of endothelial cells. MCT significantly reduced RECA-1 protein expression compared with that in the control group. Administration of CZ resulted in an improvement in the RECA-1-positive protein expression area. g–i: Immunohistochemistry of CD34. (g) Control, (h) MCT, and (i) MCT + CZ groups. CD34 protein expression in the MCT group significantly increased compared with that in the control group. Administration of CZ resulted in reduction of CD34 expression compared with that in the MCT group. j–l: Immunohistochemistry of CD41. (j) Control, (k) MCT, and (l) MCT + CZ groups. CD41-positive protein expression areas in the control group were absent. In the MCT group, CD41 protein expression was observed around the central vein and in contact with hepatocytes. In the MCT + CZ group, CD34 expression was weak compared with that in the MCT group. m–o: Immunohistochemistry of P-selectin. (m) Control, (n) MCT, and (o) MCT + CZ groups. In the control group, expression of P-selectin was weak. MCT increased the P-selectin expression area. Administration of CZ resulted in improvements in P-selectin-positive protein expression areas. p–r: Immunohistochemistry of caspase-3. (p) Control, (q) MCT, and (r) MCT + CZ groups. Decreased numbers of cells positive for caspase-3 labeling in the MCT + CZ group was observed compared with that in the MCT group. Original magnification ×400.

proliferation, degeneration and necrosis of hepatic cells, and collagen accumulation in the extracellular matrix.[25–28] It is thought that such disruption leads to portal hypertension and thrombocytopenia resulting from hypersplenism. However, the mechanisms behind these phenomena are not well understood, and an effective method to prevent and treat SOS is required.

Because platelets have no nucleus, they are not able to be observed with HE staining upon histological examination. In a previous study, we noted platelets in contact with hepatocytes of an immunosuppressed severe SOS patient[18] and in liver tissue treated with oxaliplatin-based chemotherapy.[17] In the latter study, we found that thrombocytopenia occurs before splenomegaly.[17] With electron microscopy, we found platelet aggregation in the space of Disse in an MCT-induced rat model of SOS.[19] It was suggested that platelets in the space of Disse are closely associated with the pathophysiology of SOS. We termed this phenomenon as EPA.[17–19] In the current study, CD41 protein expression, for assessment of platelet aggregation, was observed in contact with hepatocytes, especially in zone 3, to a greater extent in the MCT group compared with that in the MCT + CZ group. P-selectin protein expression, for assessment of activated platelets and damaged vascular endothelial cells,[28] was also greater in the MCT group. The platelet count significantly decreased in the MCT group, yet this decrease was significantly suppressed in the MCT + CZ group

Table 2 Sinusoidal obstruction syndrome scores for HE staining at 48 h after MCT administration

Score (range)	MCT	MCT + CZ
Sinusoidal dilatation (0–3)	2.7 ± 0.5	1.6 ± 0.7*
Necrosis of hepatocytes (0–3)	2.8 ± 0.4	1.7 ± 0.8*
Endothelial damage of the central vein (0–3)	2.7 ± 0.5	1.7 ± 0.8*
Sinusoidal hemorrhage (0–3)	2.6 ± 0.5	1.7 ± 0.9*
Total score (0–12)	10.8 ± 1.0	6.7 ± 2.1*

*$P < 0.01$ compared with MCT.

To evaluate the degree of sinusoidal obstruction syndrome, histological changes of four parameters were examined using a scoring system. The mean and total score for the monocrotaline (MCT) group were significantly greater than those in the MCT + cilostazol (CZ) group. Data are mean ± standard deviations ($n = 10$).

($P = 0.004$). This suggested that EPA were decreased by CZ administration, and SOS is strongly associated with EPA. Several recent studies using MCT-induced rat models of SOS[5,21,29,30] have suggested possibilities for prevention and/or treatment of SOS. However, these studies did not consider the influence of platelets in SOS.

The liver is an efficient regenerative organ. Numerous studies have examined the mechanisms behind liver regeneration and have found that regeneration is regulated by the release and activation of several growth factors. Fibrinolytic factors, such as hepatocyte growth factor and plasminogen activator, are considered key to liver regeneration. Several factors that inhibit the fibrinolysis system impair liver regeneration.[31–35] Among these factors, PAI-1 is a major physiological negative regulator of fibrinolytic factors. It is reported that PAI-1 interferes with liver regeneration by suppressing the hepatocyte growth factor.[36,37] Recently, PAI-1 expression was found to be significantly elevated in mice with FOLFOX-induced SOS[6] and in a rat model of hepatectomy.[36,38] PAI-1 is a well-known endothelial injury marker, but significant amounts of PAI-1 also exist in platelets. Therefore, it was suggested that PAI-1 was released following damage to LSEC and/or activated platelets.[39]

We hypothesize that if LSEC are disrupted by drugs, activated platelets migrate to the space of Disse and aggregate with collagen produced by hepatic stellate cells. We consider that thrombocytopenia occurs at this stage, on the basis of evidence from recent reports.[17,19] Sinusoid function depression and hepatic microcirculation disturbances occur and result in splenomegaly and thrombocytopenia, and, ultimately, liver dysfunction and hepatocyte necrosis occur. We suggest that EPA caused by LSEC damage may play an important role in the development of SOS, and CZ administration to strengthen LSEC and prevent EPA appeared to improve SOS.

Rat endothelial cell antigen 1 expression reflects damage to LSEC, and the observed reduction in RECA-1 indicated that the sinusoidal lining had largely disappeared. CD34 expression reflects sinusoidal capillarization in liver tissue and can be used to differentiate between normal and damaged sinusoidal epithelium.[28,40,41] In the MCT + CZ group, serum HA was significantly lower ($P = 0.039$) than that in the MCT group. As there are the large quantities of HA in LSEC, this increase in HA reflects injury to LSEC.[42,43] These results indicated that CZ attenuated MCT-induced damage to LSEC. CZ administration appeared to attenuate the macroscopic findings; elevations in serum AST, ALT, and T-Bil; and scores for HE staining. These results demonstrate the

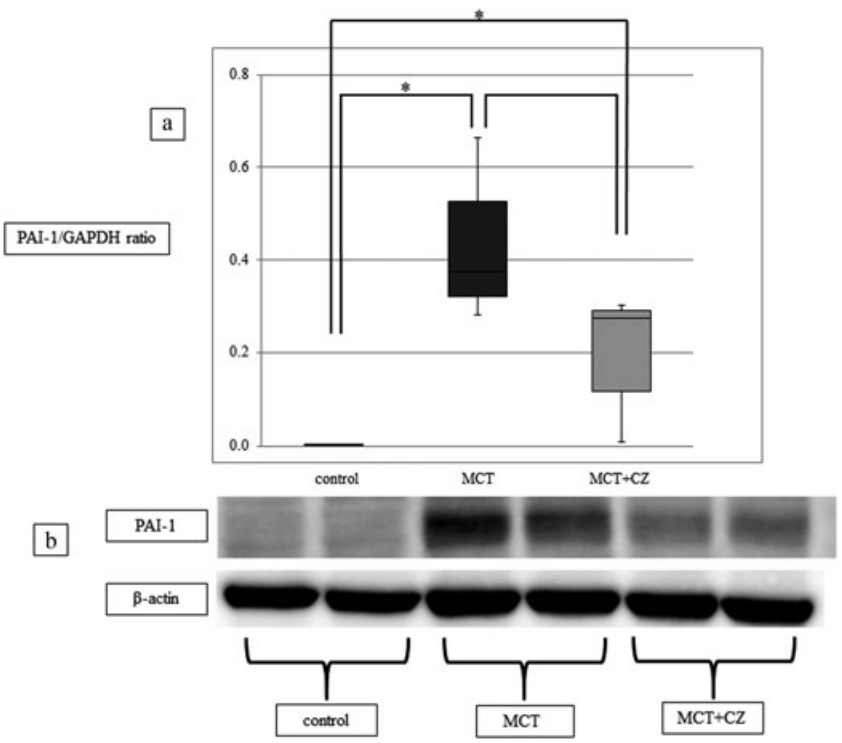

Figure 5 Expression of plasminogen activator inhibitor-1 (PAI-1). (a) Measurement of PAI-1 mRNA. PAI-1 RNA expression in the monocrotaline (MCT) and MCT + cilostazol (CZ) groups significantly increased compared with that in the control group. Although there was no significant difference, CZ resulted in a decrease in PAI-1 mRNA expression. Data are mean ± standard deviations ($n = 5$). (b) Western blotting of PAI-1. Western blots of PAI-1 and β-actin in liver tissue. Quantification of PAI-1 levels in the MCT group showed overexpression. A reduction was observed in the MCT + CZ group. *$P < 0.01$ *versus* control.

effects of CZ on SOS by protecting LSEC and suppression of the platelet aggregation in the space of Disse.

Plasminogen activator inhibitor-1 expression in the MCT and MCT + CZ groups was significantly higher than that in the control group, which was barely observed with western blotting and reverse transcription quantitative PCR analysis. The MCT + CZ group showed a decreased tendency compared with the MCT group. Hepatocyte apoptosis decreased in the MCT + CZ group, as seen with immunohistochemistry of cleaved caspase-3. We suggest that it might be possible for therapy aimed at protecting LSEC and preventing EPA with CZ administration to decrease the release of PAI-1 from LSEC and platelets and improve liver regeneration.

In the present study, we performed preventive and therapeutic CZ administration, before and after administration of MCT, and gave a significant improvement in the MCT-induced rat model of SOS. We used aspirin in the same protocol as an alternative to CZ, but the favorable results were not observed (data not shown). It is thought that aspirin can inhibit platelet aggregation but cannot suppress damage to LSEC. Previous report[5] also indicated that pretreatment with PDE-III inhibitor has potential strategy for the prevention of SOS. Actually, once SOS occurs clinically, the liver damage persists for several months.[44] These results suggest that preventive CZ administration is more effective for SOS therapy than for therapeutic CZ administration; therefore, further examination of CZ effect on SOS will be necessary.

Cilostazol, a specific inhibitor of PDE-III, inhibits platelet aggregation by increasing the cyclic adenosine monophosphate level. CZ also initiates a decrease in platelet aggregation and secretion in response to platelet agonists[45] and has been reported to suppress the expression of some adhesion molecules on the endothelium, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1.[46] These effects result in the suppression of leukocyte–endothelial interactions through modulation of eicosanoid production.[47] Several studies have reported that PDE-III inhibitors have protective effects on vasodilator action and improve vascular smooth muscle cell proliferation.[48] In particular, CZ has protective effects on LSEC by reducing inflammation by up-regulating endothelial nitric oxide synthase (eNOS) in the liver.[49] We found that CZ administration suppressed damage to LSEC and EPA and contributed to the favorable change in this MCT-induced rat model of SOS. However, CZ is not yet to be used for SOS treatment in humans induced by various anti-cancer chemotherapeutic agents, and the interaction between these drugs has not become clear. And the effect of CZ on cancer cells remains unknown. Further examination of CZ is necessary to determine its full impact before clinical application in patients with SOS.

In a recent study, an MCT-induced rat model of SOS is used to reflect human SOS. It is said that MCT rat model has the same histological liver characteristics as human SOS as well as similar symptoms.[14] However, this MCT model has some limitations. For example, the development of SOS in response to MCT is an acute event occurring within hours, whereas human SOS, induced by drugs such as oxaliplatin, occurs as a consequence of chronic drug exposure. Because of reasons like these, correlations between the MCT-induced rat model of SOS and human SOS are exactly unclear. Robinson *et al*.[6] recently reported that they had developed the first reproducible model of chemotherapy of FOLFOX-induced SOS that reflects the pathogenesis of human SOS. The further investigation of CZ against SOS is required in other models.

In conclusion, the results of the study indicated an important and novel role of CZ in this MCT-induced rat model of SOS. Prophylactic administration of CZ prevented EPA resulting from damage to LSEC. CZ administration also significantly decreased PAI-1 expression. We consider that the result promoted liver regeneration by inhibiting the coagulation system, and these changes could result in the suppression of hepatocyte apoptosis. CZ shows therapeutic potential for the prevention of SOS.

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