Transpl Int (2002) 15: 623–629
DOI 10.1007/s00147-002-0485-y

ORIGINAL ARTICLE

Kazuyuki Mizunuma
Hideki Ohdan
Hirotaka Tashiro
Yasuhiro Fudaba
Hisao Ito
Toshimasa Asahara

ROCK inhibitor Y-27632 prevents primary graft non-function caused by warm ischemia/reperfusion in rat liver transplantation

Received: 9 October 2001
Revised: 5 June 2002
Accepted: 8 July 2002
Published online: 1 November 2002

K. Mizunuma · H. Ohdan (✉)
H. Tashiro · Y. Fudaba · T. Asahara
Second Department of Surgery,
Faculty of Medicine, Hiroshima University,
1-2-3 Kasumi Minami-ku,
Hiroshima 734-8551, Japan
E-mail: mhkohdan@hotmail.com
Tel.: +81-82-2575222
Fax: +81-82-2575224

H. Ito
First Department of Pathology,
Faculty of Medicine, Tottori University,
Yonago, Japan

Abstract Hepatic stellate cells (HSCs) can easily be activated by ischemia/reperfusion, and this activation results in hepatic microcirculatory disturbance by cell contraction. ROCK is one of the key regulators of the motility of HSCs, and Y-27632 suppresses the activation of HSCs. We examined whether Y-27632 treatment prevents primary graft non-function caused by 45-min warm ischemia in orthotopic liver transplantation (OLT). Donor and recipient rats were administered Y-27632 (3–30 mg/kg). Y-27632 treatment at 30 mg/kg in both donor and recipient prevented congestion of the grafted livers, as demonstrated by analysis of hemoglobin (Hb) content in the grafted livers, using in-vivo near-infrared spectroscopy. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and hyaluronic acid at 4 h after OLT in the 30-mg/kg Y-27632-treated group were significantly lower than those in the control group. Specimens from the untreated control recipients showed sinusoidal congestion and massive fresh hepatocyte necrosis, whereas specimens from the Y-27632–treated recipients demonstrated minimal histological changes. Moreover, Y-27632 pre-treatment dramatically improved the survival of recipients. These results suggest that Y–27632 would be clinically useful for preventing liver failure associated with ischemia/reperfusion in liver transplantation.

Keywords ROCK inhibitor · Primary graft non-function · Ischemia/reperfusion · Liver transplantation · Hepatic stellate cells · Endothelin

Introduction

Non-heart-beating donors (NHBDs) are a potential source of organs for transplantation that would help alleviate the problem of donor shortage in clinical liver transplantation. However, warm ischemia/reperfusion might put recipients at risk for primary poor function or even non-function of the graft. Revolutionary progress in the prevention of ischemia/reperfusion injury may not only improve the results of transplantation, but also widen donor acceptance criteria.

Tissue injury caused by warm ischemia/reperfusion arises from the acute generation of reactive oxygen species subsequent to reoxygenation [11, 21] that directly cause damage to tissue and initiate a cascade of deleterious cellular responses leading to inflammation, cell death, and organ failure [3]. In addition to this common mechanism in all solid organs, hepatic ischemia/reperfusion injury includes peculiar pathogeneses associated with sinusoid-related cells. Kupffer cells, resident macrophages of the liver, are involved in hepatic ischemia/reperfusion injury through the release of cytokines such

as tumor necrosis factor (TNF)-α, reactive oxygen species including nitric oxide, and other inflammatory mediators [8, 26, 30]. TNF-α released from activated Kupffer cells induces endothelin-1 (ET-1) production by the vascular endothelium. ET-1 may play a role in hepatic polymorphonuclear leukocyte (PMN) infiltration as well as microcirculatory disturbance during hepatic ischemia/reperfusion injury [17].

Although there has been extensive investigation of the responses of Kupffer cells, endothelial cells, and PMNs to liver ischemia/reperfusion, little is known about the role of hepatic stellate cells (HSCs) in ischemia/reperfusion injury. HSCs are located below the sinusoidal lining in the space of Disse, and the cytoplasm of these cells contains contractile proteins such as actin and myosin [34]. It has been suggested that HSCs undergo contraction or relaxation in response to certain stimuli and, as a result, regulate hepatic microcirculation by increasing or decreasing the diameter of the sinusoidal lumen [31]. Since ET-1 is a potent vaso-active peptide synthesized by sinusoidal endothelial cells, it is likely that HSCs are easily contracted by ET-1 through ischemia/reperfusion, resulting in disruption of the hepatic microcirculation.

The Rho family of small GTPases is known to regulate cell shape and motility through reorganization of actin cytoskeletons [7]. One of the putative Rho target proteins is a serine/threonine kinase, p160ROCK [16]. Y-27632 [(+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl)-cyclohexane carboxamide dihydrochloride monohydrate] is used as a specific inhibitor of the Rho-associated coiled coil-forming protein serine/threonine kinase (ROCK) family of protein kinases and inhibits the kinase activity of both ROCK-I and ROCK-II in vitro [10]. A recent study has shown that ROCK is one of the key regulators of HSC motility and that Y-27632 suppresses ET-1-induced contraction of these cells [14]. In the present study, we investigated the capacity of Y-27632 to attenuate ischemia/reperfusion-induced disruption of the hepatic microcirculation in a rat orthotopic liver transplantation model.

Materials and methods

Animals

Male Wistar rats (Seac Yoshitomi, Fukuoka, Japan) weighting 230–280 g were used as both donors and recipients to avoid immunological influence. Animals were allowed free access to water and standard laboratory chow ad libitum. All animal experiments were performed according to the guidelines set by the National Institutes of Health (NIH publication No. 86—23, revised 1985).

Orthotopic liver transplantation

Orthotopic liver transplantation (OLT) was performed by the cuff technique as described by Kamada and Calne [13]. Briefly, under ether anesthesia, the liver of the donor was gently skeletonized. After injection of 200 IU heparin sodium, each donor rat was euthanized by deep anesthesia with ether to induce warm ischemia (at room temperature). Forty-five minutes later, the liver was flushed with chilled lactated Ringer's solution through the portal vein. After preparation, the liver was transplanted orthotopically into the recipient by supra-hepatic vena cava anastomosis with running sutures, and both the portal vein and infra-hepatic vena cava were anastomosed by the cuff technique. The hepatic artery was not reconstructed. Animals that survived more than 7 days were considered survivors.

Experimental design

Donor and recipient rats were administered Y-27632 at a dose of 3, 10, or 30 mg/kg or a control vehicle 2 h before transplantation. Recipients were further given the same doses of Y-27632 as those in the first administration 12 h after OLT. Y-27632 was kindly donated by the Welfide Corporation, Osaka, Japan.

In-vivo near-infrared spectroscopy

Our group and others have reported that in-vivo near-infrared (NIR) spectroscopy enables non-destructive evaluation of hemoglobin (Hb) oxygenation in living organ tissues [12, 19, 20]. In-vivo NIR spectroscopy was carried out as follows: NIR reflectance was measured with a multi-channel photodetector (MCPD-2000, Otsuka Electronics, Osaka, Japan). NIR light from a halogen lamp at 300 W was directed through a flexible bundle of quartz optical fibers to the liver, and the reflected light was conveyed through another bundle to the spectrophotometer. The tips of the transmitting and receiving optical fiber bundles were vertically applied to the liver and were fixed at a position approximately 3 mm above the liver. The distance between the two fiber bundles was 3 mm, so that the mean light path length, (i.e., spectrophotometric area) was theoretically 12–18 mm in diameter [29]. The reflected light was measured sequentially in the range of 500–1,100 nm at intervals of 5–10 min. During the measurement, the respiratory and heart rates were kept normal, at approximately 80 breaths/min and 200 beats/min, respectively. The difference between the spectrum from the liver after reperfusion and that from the intact liver before euthanasia was subjected to multi-component analysis. Within the range of 700–1,000 nm, the difference between the spectra was analyzed by a curve-fitting technique based on the least-squares method using standard spectra of purified oxy-Hb, deoxy-Hb, oxidized cytochrome oxidase (Cyt.aa3) and water. The five components were fitted with the following equation:

$$\begin{aligned} OD(\lambda) = L(\lambda)\cdot(e_1(\lambda)\cdot\Delta[\text{oxy-HB}] + e_2(\lambda)\cdot\Delta[\text{deoxy-Hb}] \\ + e_3(\lambda)\cdot\Delta[\text{oxidized Cyt.aa}_3] \\ + e_4(\lambda)\cdot\Delta[\text{reduced Cyt.aa}_3] + e_5(\lambda)\cdot\Delta[\text{water}]) \end{aligned}$$

where $OD(\lambda)$, $L(\lambda)$, and $e_{1-5}(\lambda)$ are optical density, mean light path length, and extinction coefficient of each component at a wavelength of λ, respectively. The relative changes in each component were detected by this multi-component analysis calculated on the basis of singular value decomposition [22].

Blood biochemical characteristics

Blood samples were collected from the inferior vena cava 4 h after transplantation, and plasma was stored at –80 °C until required for analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and hyaluronic acid were assayed by standard enzymatic methods. Plasma endothelin levels were measured with an ET-1 assay kit (IBL, Fujioka, Japan) according to the manufacturer's instructions.

Histological examination

Liver specimens were obtained 4 h after transplantation in the 30-mg/kg Y-27632-treated group and the control group. Samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin. The fixed samples were sectioned transversely and stained with hematoxylin and eosin for standard microscopic examination.

Statistical analysis

The results are expressed as means ± SD. Differences between two groups were analyzed by the unpaired Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Results

Survival of liver-transplanted rats

The 7-day survival rates of recipient rats with liver grafts that had been subjected to 45-min warm ischemia were 11.1% (1/9) in the control group, 0% (0/5) in the 3-mg/kg Y-27632-treated group, 20% (1/5) in the 10-mg/kg Y-27632-treated group, and 88.9% (8/9) in the 30-mg/kg Y-27632-treated group (Fig. 1). When the donors were treated with the control vehicle, eight of the nine recipients died of primary graft non-function within 3 days after OLT, indicating a lethal condition in this control group. In contrast, recipient survival was dramatically improved in the 30-mg/kg group, demonstrating that treatment of Y-27632 prevented ischemia/reperfusion injury. The 7-day survival rate after OLT was significantly higher in the 30mg/kg Y-27632-treated group than in the control group ($P = 0.0009$; log-rank test).

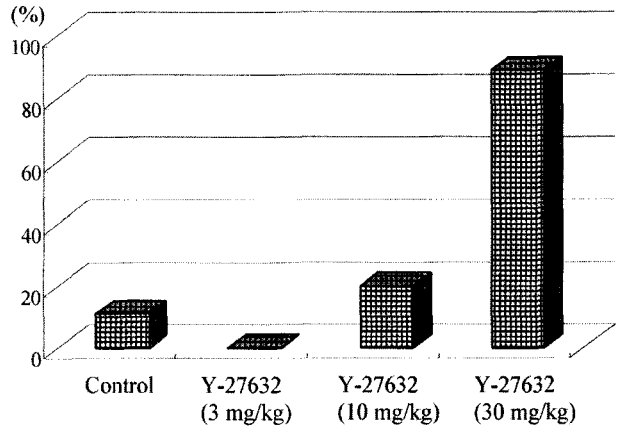

Fig. 1 Seven-day survival rates of recipient rats with liver grafts that had been subjected to 45-min warm ischemia. Donor and recipient rats were administered Y-27632 at a dose of 3, 10, or 30 mg/kg or a control vehicle 2 h before transplantation. Recipients were further given the same doses of Y-27632 as those in the first administration 12 h after OLT. Numbers of animals in each group: untreated control group, $n = 9$; Y-27632–treated group (3 mg/kg), $n = 5$; Y-27632-treated group (10 mg/kg), $n = 5$; Y-27632-treated group (30 mg/kg), $n = 9$. The 7-day survival rate after transplantation was significantly higher in the 30 mg/kg Y-27632-treated group than in the control group ($P = 0.0009$; log-rank test)

NIR analysis for evaluating hepatic congestion

After OLT, changes in total, oxy-, and deoxy-Hb contents in the liver tissues were monitored by NIR spectroscopy. In untreated control rats, the liver tissue contents of total Hb persistently increased after reperfusion, indicating hepatic congestion (Fig. 2a). The congestion was due to

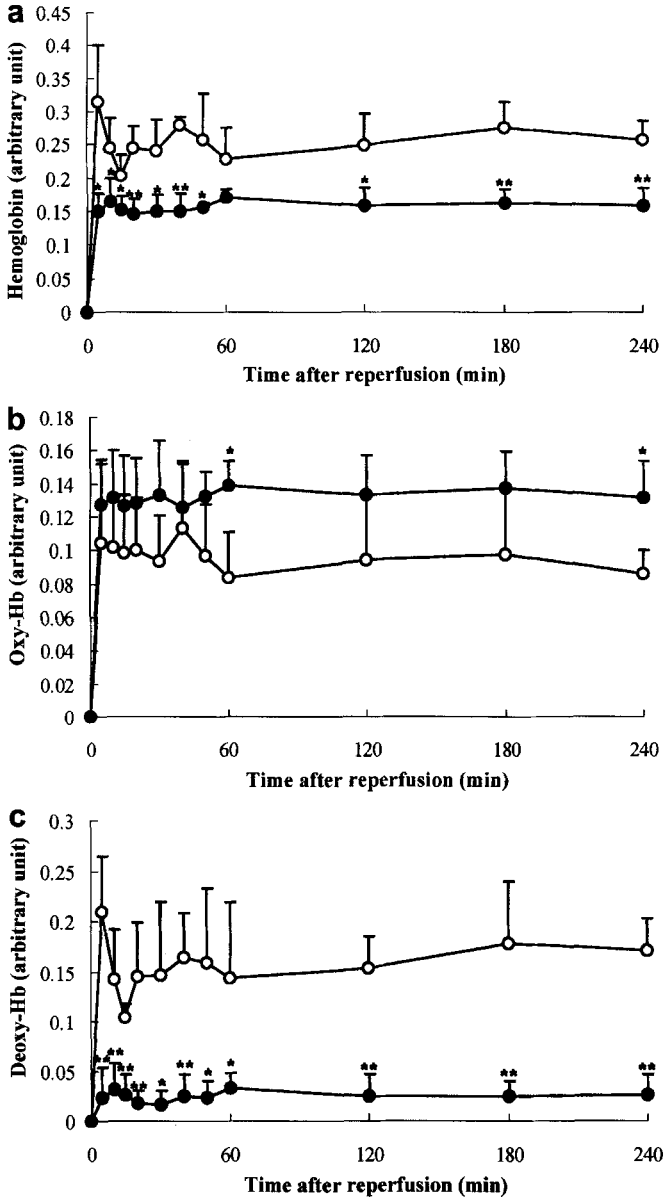

Fig. 2a–c Changes in total, oxy-, and deoxy-Hb content in liver tissues after transplantation following 45 min of warm ischemia (at room temperature) in donor rats. After transplantation, liver tissue content of Hb was determined by in-vivo NIR spectroscopy. Kinetics of total Hb (**a**), oxy-Hb (**b**), and deoxy-Hb (**c**) content in liver tissues are shown. Data are shown as average values ± SD for individual groups. $*P < 0.05$ vs control, $**P < 0.01$ vs control. *Open circles* untreated control rats ($n = 4$), *closed circles* rats that received 30 mg/kg of Y-27632 ($n = 4$)

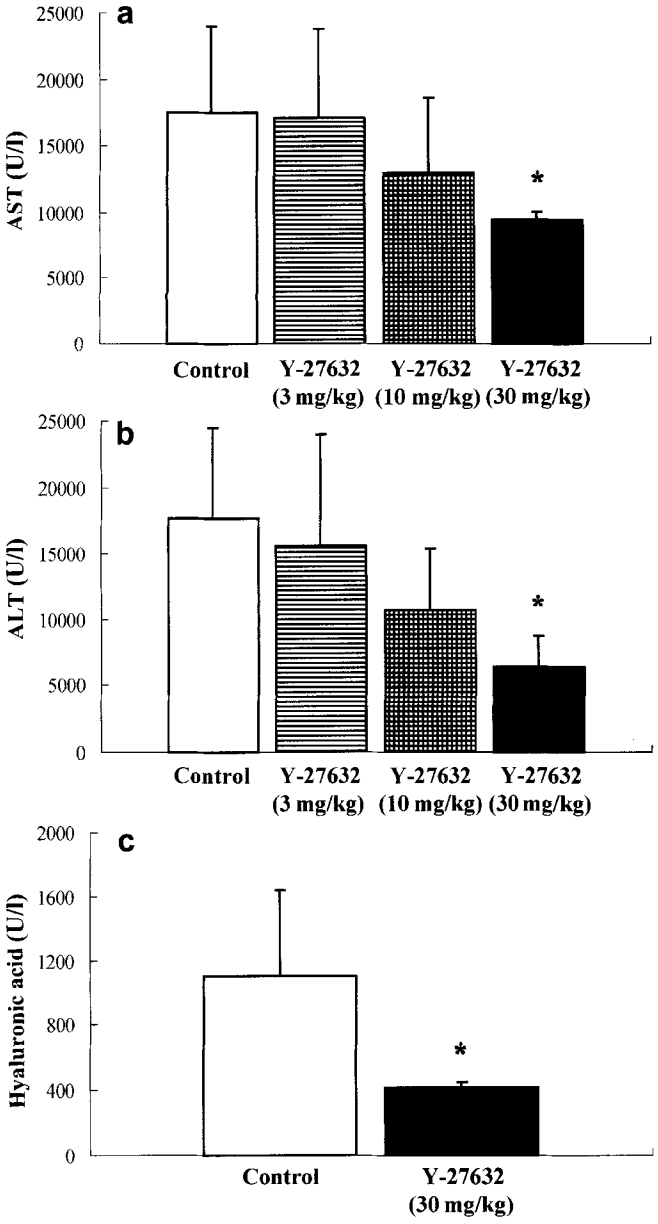

Fig. 3a–c Plasma-releasing enzyme and plasma hyaluronic acid concentration 4 h after OLT following 45 min of warm ischemia. Y-27632 treatment (30 mg/kg) significantly suppressed the elevations of AST (**a**), ALT (**b**), and hyaluronic acid (**c**). Average values ± SD for individual groups are shown. $*P < 0.05$ vs control rats. Numbers of animals in each group: untreated control group, $n = 9$; Y-27632-treated group (3 mg/kg), $n = 5$; Y-27632-treated group (10 mg/kg), $n = 5$; Y-27632-treated group (30 mg/kg), $n = 9$

accumulation of both oxy-Hb and deoxy-Hb (Fig. 2b and c). In contrast, in Y-27632-treated (30 mg/kg) rats, the elevation of total Hb content in the liver was significantly suppressed after reperfusion. Notably, deoxy-Hb content in the liver was not altered, even after reperfusion. Thus, Y-27632 treatment prevented hepatic congestion induced by ischemia/reperfusion in OLT.

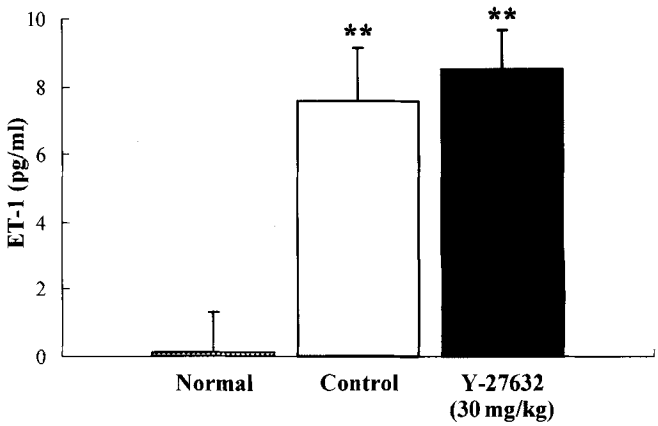

Fig. 4 Plasma ET-1 concentration 4 h after OLT. Marked elevation of plasma ET-1 was observed in both untreated control and Y-27632-treated rats. $**P < 0.01$ vs normal rats. Numbers of animals in each group: normal rats, which were not subjected to liver ischemia, $n = 6$; untreated control group, $n = 6$; Y-27632-treated group (30 mg/kg), $n = 6$

Levels of released enzymes in plasma after OLT

Figure 3a, b show plasma enzyme data that reflect liver functions at 4 h after reperfusion. Y-27632 treatment significantly suppressed the elevations of AST and ALT in a dose-dependent manner ($P < 0.05$, 30-mg/kg-treated vs untreated). In addition, it also suppressed the elevation of plasma hyaluronic acid level, a possible indicator of sinusoidal endothelial cell injury (Fig. 3c) ($P < 0.05$, 30-mg/kg-treated vs untreated).

Plasma ET-1 levels after OLT

Figure 4 shows the plasma ET-1 concentration, which reflects activation of sinusoidal endothelial cells, at 4 h after reperfusion. Marked elevation in plasma ET-1 was observed in untreated control recipients of OLT when compared with that in normal naive rats. There were no significant differences between the ET-1 levels in the 30-mg/kg treated group and the untreated control group, suggesting that Y-27632 did not directly affect endothelial cells.

Pathological findings

The grafted liver specimens obtained from the untreated control group at 4 h after OLT showed sinusoidal congestion and multiple and extensive areas of fresh hepatocyte necrosis, indicating ischemia/reperfusion injury (Fig. 5a). On the other hand, the grafted liver specimens from the 30-mg/kg Y-27632-treated group showed minimal histological changes of hepatic structure and

sinusoidal enlargement in the hepatic lobule center compared with that in untreated control rats (Fig. 5b).

Discussion

HSCs have many cytoplasmic processes and surround hepatic endothelial cells. The main functions of HSCs are vitamin A storage [5] and the production of various extracellular matrix components [2]. The morphology of the hepatic sinusoidal wall resembles that of a capillary. HSCs, which correspond to pericytes around capillaries, are localized in the space of Disse around the hepatic sinusoids. Pericytes contain contractile proteins such as actin and myosin, and these cells are involved in the control of capillary blood flow. These facts together with the fact that HSCs contain calmodulin (Ca^{2+}-binding protein) as well as actin and myosin [27] suggest that HSCs are involved in the regulation of blood flow in the hepatic sinusoids by contraction and relaxation [24].

A recent study has shown that ROCK is one of the key regulators of HSC motility and that Y-27632 suppresses ET-1-induced contraction of these cells [14]. Rho A, a small monomeric G protein, enhances sensitivity to Ca^{2+} by affecting myosin light-chain processes in smooth muscle [4, 6]. ROCK, which is a target protein of Rho A [9, 15], inhibits myosin light-chain phosphatase activity. It has been revealed that Y-27632, a selective ROCK inhibitor, effectively decreases Ca^{2+} sensitivity mediated by various agonists, including ET-1 [28]. Such activities of Y-27632 suggest that HSCs might undergo relaxation in the presence of ET-1, through a decrease in Ca^{2+} sensitivity rather than a decrease in intercellular Ca^{2+} concentration. Considering the fact that ET-1 increases cellular tension even at a constant intercellular Ca^{2+} concentration (a phenomenon referred to as Ca^{2+} sensitization) [18, 25], Y–27632 seems to be superior to Ca-channel blockers, which are known to prevent constriction in the sinusoids by inhibiting intercellular Ca^{2+} influx, in ameliorating hepatic ischemia/reperfusion injury.

Endothelin, a potent vaso-active peptide synthesized by endothelial cells, induces contraction of perivascular smooth muscle cells by enhancing their Ca^{2+} sensitivity [18]. It is well known that hepatic ischemia/reperfusion stimulates activity of sinusoidal endothelial cells, facilitating their production of ET-1 [33]. Taken together with the fact that ET receptors are most abundant on HSCs among all hepatic cell types [23], it is likely that contraction of HSCs causes hepatic microcirculatory disturbance after ischemia/reperfusion through the binding of ET-1 and endothelin receptors on HSCs. In this study, we demonstrated that Y-27632 treatment did not reduce ET-1 production (Fig. 4), but attenuated hepatic microcirculatory disturbance after ischemia/reperfusion in OLT.

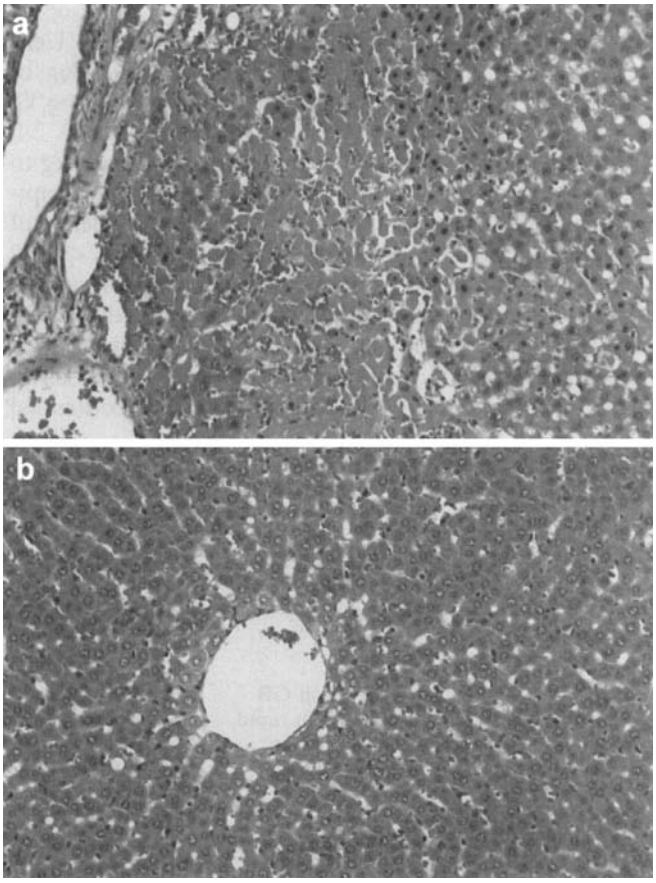

Fig. 5a, b Histology of the grafted liver 4 h after OLT. **a** Liver specimens from untreated control rats showed sinusoidal congestion and multiple and extensive areas of fresh hepatocyte necrosis. **b** Liver specimens from the 30-mg/kg Y-27632-treated rats showed minimal histological change of hepatic structure (original magnification, × 200)

In addition to the inhibitory effect of Y-27632 on the contraction of HSCs, this compound might have a beneficial effect on infiltration of PMNs and monocytes, which also contributes to hepatic ischemia/reperfusion injury. This possibility has been suggested by the results of recent studies showing that Rho A regulates the motility of PMNs and trans-endothelial migration of monocytes [1, 32]. Adhesion and migration of PMNs and monocytes after ischemia/reperfusion could be associated with aggravation of injury in the late phase. This might have caused the late deaths of untreated rats that underwent transplantation. It is possible that the beneficial effect of Y-27632 on the survival of rats receiving liver grafts that had been exposed to warm ischemia might involve action against infiltration of PMNs and monocytes in addition to action against contraction of HSCs. This possibility is currently under investigation.

From the viewpoint of clinical application, the optimal dose, timing, and period of Y-27632 treatment must

be addressed. Based on the pharmacokinetic data of Y-27632 provided by the Welfide Corporation (Dr. Uehata), Y-27632 was given 2 h before the harvesting of graft livers in the donor rats. By the giving of 30 mg/kg of Y-27632 to both the donor and recipient of OLT 2 h before ischemic exposure and an additional 30 mg/kg to the recipient 12 h after OLT, the survival of the recipients was significantly improved without any evidence of side effects (Fig. 1). Although the details of Y-27632 metabolism in the liver remain to be elucidated, such a regimen of this compound seems to be clinically applicable for preventing liver failure associated with ischemia/reperfusion. However, donor pre-treatment before warm ischemia would be difficult in an uncontrolled situation. Further efforts to dispense with donor pretreatment must be made in order to meet a more liberal donor policy.

In conclusion, Y-27632 attenuates ischemia/reperfusion-induced hepatic microcirculation disruption probably by modulating activation of HSCs, and this compound would be a clinically useful tool for the prevention of primary graft non-function associated with warm ischemia/reperfusion in liver transplantation.

Acknowledgements This study was supported in part by a grant-in-aid for Research on Human Genome, Tissue Engineering, Food Biotechnology, Health Sciences from the Ministry of Health, Labor, and Welfare of Japan.

References

1. Anderson SI, Hotchin NA, Nash GB (2000) Role of the cytoskeleton in rapid activation of CD11b/CD18 function and its subsequent downregulation in neutrophils. J Cell Sci 113:2737–2745
2. Clement B, Grimaud JA, Campion JP, Deugnier Y, Guillouzo A (1986) Cell types involved in collagen and fibronectin production in normal and fibrotic human liver. Hepatology 6:225–234
3. Fan C, Zwacka RM, Engelhardt JF (1999) Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med 77:577–592
4. Fujihara H, Walker LA, Gong MC, Lemichez E, Boquet P, Somlyo AV, Somlyo AP (1997) Inhibition of Rho A translocation and calcium sensitization by in vivo ADP-ribosylation with the chimeric toxin DC3B. Mol Biol Cell 8:2437–2447
5. Hendriks HF, Verhoofstad WA, Brouwer A, de Leeuw AM, Knook DL (1985) Perisinusoidal fat-storing cells are the main vitamin storage sites in rat liver. Exp Cell Res 160:138–149
6. Hirata K, Kikuchi A, Sasaki T, Kuroda S, Kaibuchi K, Matsuura Y, Seki H, Saida K, Takai Y (1992) Involvement of rho p21 in the GTP-enhanced calcium ion sensitivity of smooth muscle contraction. J Biol Chem 267:8719–8722
7. Hirose M, Ishizaki T, Watanabe N, Uehata M, Kranenberg O, Moolenaar WH, Matsumura M, Maekawa M, Bito H, Narumiya S (1998) Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. J Cell Biol 141:1625–1636
8. Hisama N, Yamaguchi Y, Ishiko T, Miyanari N, Ichiguchi O, Goto M, Mori K, Watanabe K, Kawamura K, Tsurufuji S, Ogawa M (1996) Kupffer cell production of cytokine-induced neutrophil chemoattractant following ischemia/reperfusion injury in rats. Hepatology 24:1193–1198
9. Ishizaki T, Maekawa M, Fujisawa K, Okawa K, Iwamatsu A, Fujita A, Watanabe N, Saito Y, Kakizuka A, Morii N, Narumiya S (1996) The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. EMBO J 15:1885–1893
10. Ishizaki T, Uehata M, Tamechika I, Keel J, Nonomura K, Maekawa M, Narumiya S (2000) Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. Mol Pharmacol 57:976–983
11. Jaeschke H (1991) Reactive oxygen and ischemia/reperfusion injury of the liver. Chem Biol Interact 79:115–136
12. Jobsis-van derVliet FF, Piantadosi CA, Sylvia AL, Lucas SK, Keizer HH (1988) Near-infrared monitoring of cerebral oxygen sufficiency. I. Spectra of cytochrome c oxidase. Neurol Res 10:7–17
13. Kamada N, Calne RY (1979) Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 28:47–50
14. Kawada N, Seki S, Kuroki T, Kaneda K (1999) ROCK inhibitor Y-27632 attenuates stellate cell contraction and portal pressure increase induced by endothelin-1. Biochem Biophys Res Commun 266:296–300
15. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okano T, Iwamatsu A, Kaibuchi K (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 273:245–248
16. Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, Narumiya S (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 285:895–898
17. Mitsuoka H, Suzuki S, Sakaguchi T, Baba S, Miwa M, Konno H, Nakamura S (1999) Contribution of endothelin-1 to microcirculatory impairment in total hepatic ischemia and reperfusion injury. Transplantation 67:514–520
18. Nishimura J, Moreland S, Ahn HY, Kawase T, Moreland RS, van Breemen C (1992) Endothelin increases myofilament Ca^{2+} sensitivity in α-toxin-permeabilized rabbit mesenteric artery. Circ Res 71:951–959
19. Noriyuki T, Ohdan H, Yoshioka S, Miyata Y, Asahara T, Dohi K (1997) Near-infrared spectroscopic method for assessing the tissue oxygenation state of living lung. Am J Respir Crit Care Med 156:1656–1661
20. Ohdan H, Fukuda Y, Suzuki S, Amemiya H, Dohi K (1995) Simultaneous evaluation of nitric oxide synthesis and tissue oxygenation in rat liver allograft rejection using near-infrared spectroscopy. Transplantation 60: 530–535
21. Poli G (1993) Liver damage due to free radicals. Br Med Bull 49:604–620

22. Press WH, Teukolsky SA, Vetterling WT, Flannery BP (1992) Numerical recipes in C: the art of scientific computing, 2nd edn. Cambridge University Press, Cambridge, pp 59–70
23. Rockey DC (1995) Characterization of endothelin receptors mediating rat hepatic stellate contraction. Biochem Biophys Res Commun 207:725–731
24. Sakamoto M, Ueno T, Kin M, Ohira H, Torimura T, Inuzuka S, Sata M, Tanikawa K (1993) Ito cell contraction in response to endothelin-1 and substance P. Hepatology 18:978–983
25. Sakata K, Ozaki H, Kwon SC, Karaki H (1989) Effects of endothelin on the mechanical activity and cytosolic calcium level of various types of smooth muscle. Br J Pharmacol 98:483–492
26. Suzuki S, Toledo-Pereyra LH, Rodriguez F, Lopez F (1994) Role of Kupffer cells in neutrophil activation and infiltration following total hepatic ischemia and reperfusion. Circ Shock 42:204–209
27. Tanikawa K, Ueno T (1991) Intrinsic innervation and Ito cells in human liver. In: Tsuchiya M, Nagura H, Hibe T, Moro I (eds) Frontiers of mucosal immunology, vol 2. Excerpta Medica, New York, pp 179–182
28. Uehata M, Ishizaki T, Sato H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, Narumiya S (1997) Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 389:990–994
29. Van der Zee P, Cope M, Arridge SR, Essenpreis M, Potter LA, Edwards AD, Wyatt JS, McCormick DC, Roth SC, Reynolds EO (1992) Experimentally measured optical pathlengths for the adult head, calf, and forearm and the head of the newborn infant as a function of inter-optode spacing. Adv Exp Med Biol 316:143–153
30. Wanner GA, Ertel W, Muller P, Hofer Y, Leiderer R, Menger MD, Messmer K (1996) Liver ischemia and reperfusion induces a systemic inflammatory response through Kupffer cell activation. Shock 5:34–40
31. Watanabe S, Wang XE, Hirose M, Oide H, Shimizu H, Kitamura T, Yumoto A, Suzuki S, Miyazaki A, Sato N (1995) Integrity of myosin light chain kinase is essential for Ito cell contraction. J Gastroenterol Hepatol 10 [Suppl 1]:S54–S56
32. Worthylake RA, Lemoine S, Watson JM, Burridge K (2001) Rho A is required for monocyte tail retraction during transendothelial migration. J Cell Biol 154:147–160
33. Yanagisawa M (1994) The endothelin system. A new target for therapeutic intervention. Circulation 89:1320–1322
34. Yokoi Y, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S, Usui K (1984) Immunocytochemical detection of desmin in fat-storing cells (Ito cells). Hepatology 4:709–714