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ORIGINAL ARTICLE

MCI-186 (edaravone), a free radical scavenger, attenuates hepatic warm ischemia–reperfusion injury in rats

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Summary

Hepatic warm ischemia–reperfusion injury (IRI) during hepatectomy and liver transplantation is a major cause of liver dysfunction in which the pathologic role of free radicals is a major concern. To assess the effect of MCI-186 (edaravone) on hepatic IRI, male Wistar rats were subjected to partial hepatic ischemia for 60 min after pretreatment with vehicle (group C) or MCI-186 (group M), or after both MCI-186 pretreatment and additional administration of MCI-186 12 h after reperfusion (group MX). Groups M and MX showed significantly lower levels of serum alanine aminotransferase and hepatic lipid peroxidation than group C, and also significantly lower expression levels of mRNA for cytokines, chemokines and intercellular adhesion molecule-1. There were fewer tissue monocytes and neutrophils in groups M and MX than in group C. These effects were more marked in group MX than in group M. Our findings suggest that treatment with MCI-186 attenuates hepatic IRI in this rat *in vivo* model.

Introduction

Warm ischemia–reperfusion injury (IRI) during hepatic resection and liver transplantation may lead to local and systemic organ dysfunction. The local hepatic injury comprises two phases, the acute (early) phase and the subacute (late) phase [1–9]. During the acute phase, Kupffer cells are activated and release oxygen-derived free radicals (ODFRs) and proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8. The subacute phase is mediated by infiltrating neutrophils that are primed and activated during the acute phase [1–9]. Chemokines released by Kupffer cells, including CXC chemokines and CC chemokines, may also play important roles in hepatic IRI. CXC chemokines induce neutrophil activation and CC chemokines activate macrophages and T cells and upregulate cell adhesion molecules [10–13]. While recent studies have shown that not only macrophages (Kupffer cells) and neutrophils but also T cells play significant roles in hepatic IRI [3,5,6,14,15], the significance of free radicals in the pathology of the acute and subacute phases of hepatic IRI is thought to be crucial.

MCI-186 [edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one); Mitsubishi Pharma Co., Osaka, Japan] is a free radical scavenger. This reagent has already been applied clinically for the prevention of brain edema in patients with acute cerebral infarction through inhibition of the lipoxygenase pathway in the arachidonic acid cascade and has shown positive results [16–19]. Several reports have described the effects of MCI-186 on hepatic IRI. In *ex vivo* hepatic IRI experiments [20,21], perfusion with Krebs–Henseleit solution cannot reproduce the effects of circulating macrophages and neutrophils, which play major roles in hepatic IRI. In *in vivo* experiments, the efficacy of MCI-186 in attenuating hepatic warm IRI has been evaluated in terms of aspartate aminotransferase, phosphatidylcholine hydroperoxide, adenosine triphosphate [22], and mitochondrial function [23]. However,

the effects of MCI-186 on monocytes, neutrophils and their associated cytokines have not been evaluated.

In the present study, we evaluated the potential of MCI-186 to attenuate hepatic warm IRI in a partial-IRI rat model *in vivo*. We focused on the changes in lipoxygenase activation, monocyte activation, chemokine expression, neutrophil infiltration and hepatic dysfunction resulting from administration of MCI-186.

Materials and methods

Experimental animals and reagents

Male Wistar rats (Clea Japan Inc., Tokyo, Japan) weighing 200–250 g were used. All animals were maintained under standard conditions and fed rodent chow and water *ad libitum*. Twelve hours before surgery, the animals were fasted, but allowed access to water. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Shinshu University.

The following monoclonal antibodies were used as primary antibodies for immunohistochemistry: HNEJ-2 (Nikken Seil Co., Shizuoka, Japan) specific for 4-hydroxy-2-nonenal (4-HNE) modified protein, ED-1 (Serotec, Oxford, UK) specific for rat CD163 expressed on free and fixed macrophages, ED-2 (Serotec) specific for rat CD68 expressed on Kupffer cells and on residential macrophages, and HIS48 (BD Biosciences, Palo Alto, CA, USA) specific for rat neutrophils.

Hepatic IRI model

All surgical procedures were carried out according to the protocol described elsewhere [24]. Rats were anesthetized with sodium pentobarbital 50 mg/kg, intraperitoneally (Dainippon Pharmaceutical Co. Ltd, Osaka, Japan). After laparotomy, a microvascular clip (BEAR Medic Co., Chiba, Japan) was used to interrupt the arterial and portal venous supply to the median and left lateral lobes of the liver. This resulted in ischemia of approximately 70% of the whole liver while avoiding portal venous congestion. After 60 min of partial hepatic ischemia, the clamp was removed for reperfusion. The abdomen was closed and 1 ml of saline was administered intravenously. The rats were killed 1, 3, 6 and 24 h after reperfusion, and blood and tissue samples were harvested for analysis.

Experimental protocol

The rats were divided into three groups(Fig. 1). Group S was a sham operation group. Group C comprised IRI model rats, administered saline 5 min before reperfusion. Group M comprised IRI model rats, administered MCI-186 (3 mg/kg) 5 min before reperfusion. Saline and MCI-186 were injected intravenously. The dose and timing of saline and MCI-186 administration were based on previous reports [25–27].

Each group was divided into four subgroups according to the time (h) from reperfusion to killing [groups S-1, S-3, S-6, S-24 ($n = 5$, respectively), C-1 ($n = 6$), C-3 ($n = 5$), C-6 ($n = 7$), C-24 ($n = 7$), M-1 ($n = 6$), M-3 ($n = 5$), M-6 ($n = 7$) and M-24 ($n = 5$)]. For groups S-24, C-24 and M-24, additional administration of saline was performed 12 h after reperfusion.

In addition, considering the short half-life of MCI-186 as well as the acute and subacute mechanisms underlying IRI, a fourth group was additionally administered MCI-186 12 h after reperfusion instead of saline, followed by killing 24 h after reperfusion [group MX-24 ($n = 7$)]. This protocol was used to evaluate the role of MCI-186 in the subacute phase of IRI.

Peripheral blood and tissue samples

Blood samples were obtained via the abdominal aorta. The blood was centrifuged (2500 *g*, 10 min) at room temperature and the plasma was collected and stored at −20 °C until use. Portions of the ischemic and nonischemic lobes were fixed in 10% buffered formalin and embedded in paraffin. Other portions were snap-frozen in

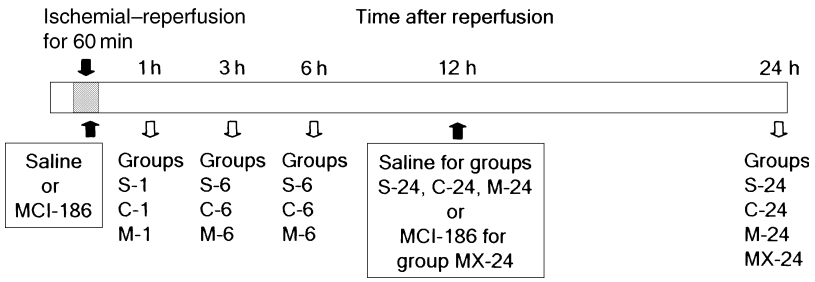

Figure 1 Experimental protocols for rat hepatic ischemia–reperfusion injury models.

liquid nitrogen to extract mRNA and embedded in optimal cutting temperature (OCT) compound (Sakura Finetechnical Co., Tokyo, Japan) for immunohistochemistry, and stored at −80 °C until used.

Measurement of serum aminotransferase

To evaluate hepatic injury, we measured serum alanine aminotransferase (ALT) levels at the time of killing in each group using an AU5232 autoanalyzer (Olympus, Tokyo, Japan), as described previously [24].

Histologic examination

Samples were fixed with 10% buffered formaldehyde and embedded in paraffin. Sections at 3-μm intervals were prepared and stained with hematoxylin and eosin for histologic examination. A blinded analysis was performed by two pathologists to determine the degree of lesions observed ($n = 5$, independent animals in each group). The degrees of sinusoidal congestion, cytoplasmic vacuolization and necrosis of parenchymal cells were evaluated semiquantitatively according to the criteria described in a previous study [28].

Immunohistochemistry

Immunohistochemical detection of lipid peroxidation and inflammatory cell recruitment was carried out. For detection of 4-HNE and macrophages, 10% buffered formaldehyde-fixed and paraffin-embedded tissue samples were cut into 3-μm-thick sections. Antigen retrieval was performed by 25 min of microwave irradiation in 1.0 mM EDTA-Na_2 for 4-HNE, and by 6 min of proteinase K digestion (0.4 mg/ml) for ED-2. No retrieval procedure was performed for ED-1. After antigen retrieval, the sections were incubated with primary antibodies at 4 °C overnight for 4-HNE and at room temperature for 60 min for ED-1 and ED-2. The dilutions of the primary antibodies were 1:80 for 4-HNE, 1:200 for ED-1 and 1:100 for ED-2. Goat anti-mouse immunoglobulin conjugated with peroxidase-labeled dextran polymer (Envision^{TM}+ system; Dako, Carpinteria, CA, USA) was used as the secondary antibody.

For detection of neutrophils, we used frozen sections. Samples embedded in OCT compound were cut into 5-μm-thick sections, placed on adhesive-coated slides (Matsunami Glass, Osaka, Japan), and then air-dried. After blocking with 1% normal goat serum in tris-buffered saline, these tissue specimens were incubated at room temperature for 60 min with a primary monoclonal antibody, HIS48. After washing with phosphate-buffered saline, the specimens were incubated using the Envision^{TM}+ system.

In a control experiment, the primary antibody was omitted from the staining procedure, and no specific staining was found. Counterstaining was carried out with hematoxylin.

Analysis of mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from tissue with ISOGEN (Nippon Gene Co. Ltd, Tokyo, Japan). cDNA was reverse-transcribed from 2 μg of total RNA using an Omniscript^{TM} Reverse Transcriptase kit (Qiagen GmbH, Hilden, Germany). The cDNA was amplified by RT-PCR using a *Taq* polymerase core kit (Qiagen GmbH). We prepared primer sets for RT-PCR as shown in Table 1, and the final reaction volume was 25 μl. The samples were loaded into a thermal cycler after determining the optimal number of cycles as follows: 30 cycles of denaturing at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min for TNF-α and IL-1β; 30 cycles at 94 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min for cytokine-induced neutrophil chemoattractant (CINC)-2 and macrophage inflammatory protein (MIP)-2; 25 cycles at 94 °C for 30 s, 60 °C for 1 min, and 72 °C

Table 1. Polymerase chain reaction primer sets for cytokines, chemokines, and adhesion molecule.

	Sense (5′ → 3′)	Anti-sense (5′ → 3′)
β-actin	CGG CAT TGT AAC CAA CAG G	CAT TGC CGA TAG TGA TGA CC
TNF-α	TAC TGA ACT TCG GGG TGA TTG GTC C	CAG CCT TGT CCC TTG AAG AGA A
IL-1β	GCT ACC TAT GTC TTG CCC GT	GAC CAT TGC TGT TTC CTA GG
CINC-2	GCT ACC TAT GTC TTG CCC GT	TGA CCA TCC TTG GAG AGT GGC
MIP-2	AGC TCC TCA ATG CTG TAC TGG	TCT ATC ACA GTG TGG AGG TGG
MCP-1	CTC TTC CTC CAC CAC TAT GC	CTC TGT CAT ACT GGT CAC TTC
MIP-1α	GAA GGA AAG TCT TCT CAG CG	AGA CAT TCA GTT CCA GC
MIP-1β	ATG AAG CTC TGC GTG TCT GC	AGT TCC GAT GAA TCT TCC GG
ICAM-1	GAT GCT GAC CCT CCA CAC CA	CAG GGA CTT CCC ATC CAC CT

TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1 beta; CINC-2, cytokine-induced neutrophil chemoattractant-2; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; ICAM-1, intercellular adhesion molecule-1.

for 1 min for monocyte chemoattractant protein (MCP)-1; 30 cycles at 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min for MIP-1α; 30 cycles at 94 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min for MIP-1β; and 30 cycles at 94 °C for 45 s, 55 °C for 30 s, and 72 °C for 90 s for intercellular adhesion molecule (ICAM)-1. The housekeeping gene β-actin was used as the RT-PCR control, and its cycling program was 25 cycles at 94 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min. For every gene, the final cycle was followed by soaking for 7 min at 72 °C. RT-PCR products were analyzed using 2.0% agarose gel electrophoresis, and visualized by staining with ethidium bromide.

Statistical analysis

Differences among the groups were evaluated by the Mann–Whitney *U*-test. All values are expressed as mean ± SEM, and data were considered significant at $P < 0.05$.

Results

Serum ALT levels at 1, 3, 6 and 24 h after reperfusion

The serum ALT levels in each group are shown in Fig. 2. Among the groups that underwent ischemia–reperfusion, ALT increased to 11207 ± 1957 U/l in group C-6, but decreased to 3782 ± 1334 U/l in group M-6, which was significantly lower than the level in group C-6. The effect of MCI-186 became less prominent 24 h after reperfusion.

Consequently, we focused on changes occurring 24 h after reperfusion. The ALT levels were 5445 ± 1155 U/l (group C-24) vs. 4405 ± 1387 U/l (group M-24). However, additional administration of MCI-186 at 12 h after reperfusion markedly inhibited the increase in ALT levels to 1065 ± 605 U/l (group MX-24).

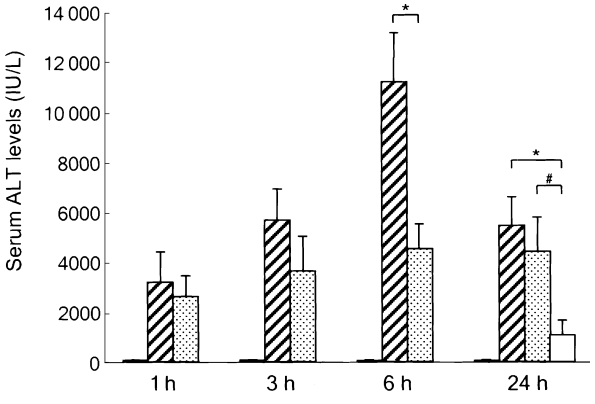

Figure 2 Effects of MCI-186 on serum alanine aminotransferase (ALT) levels. In the acute phase, rats treated with MCI-186 (group M) had lower ALT levels than the saline-treated group (group C). The ALT level in group MX-24 was significantly lower than that in groups C-24 and M-24. Values are expressed as mean ± SEM. *$P < 0.05$ compared with group C; #$P < 0.05$ compared with group M-24. ■, group S; ▨, group C; ▩, group M; □, group MX.

Histologic analysis

No pathologic findings were observed in the liver tissues of the sham control groups (data not shown). In group C-6, liver specimens exhibited focal necrosis, sinusoidal congestion and infiltration of leukocytes, and these changes became more severe in group C-24 (Fig. 3). In contrast, such findings were minimal in group M-6, but were observed in group M-24. In group MX-24, pathologic findings of spotty necrosis, sinusoidal congestion and infiltration of leukocytes were minimal compared with groups C-24 and M-24. These results were confirmed by a semi quantitative assessment, and shown to be significant (Table 2).

Immunohistochemistry for 4-HNE detection

There were no 4-HNE-positive cells in the liver in the sham control groups (data not shown). In groups C-6 and C-24, 4-HNE-positive cells were observed, but the 4-HNE levels were demonstrably reduced in group M-6 (Fig. 4). The number of 4-HNE-positive cells was lower in group MX-24 than in groups C-24 and M-24.

Immunohistochemistry for infiltrating cells

The numbers of cells positive for ED-1 (free macrophages) and ED-2 (resident macrophages) in the ischemic lobes were increased in group C-6, but not in group M-6 (Fig. 5a and b). However, such cells were increased to some extent in group M-24. In group MX-24, the numbers of both ED-1 and ED-2 positive cells were markedly reduced in comparison with groups C-24 and M-24. Figure 5c shows a similar tendency of infiltrated neutrophils into the sinusoids.

Expression of cytokine and chemokine mRNAs

Expression of cytokine and chemokine genes in the ischemic hepatic lobes in groups C and M was compared with that in group S, the sham control group (Fig. 6a). Expression of TNF-α mRNA was high after 1 h (group C-1) and then gradually decreased with time, and was lower in group M-1 than in group C-1 (Fig. 6b). Expression of IL-1 mRNA was high in groups C-1, C-3 and C-6, but attenuated in groups M-3 and M-6. The expression levels of CINC-2 (rat IL-8), MIP-2, MCP-1, MIP-1α and MIP-1β mRNAs were increased in groups C-3 and

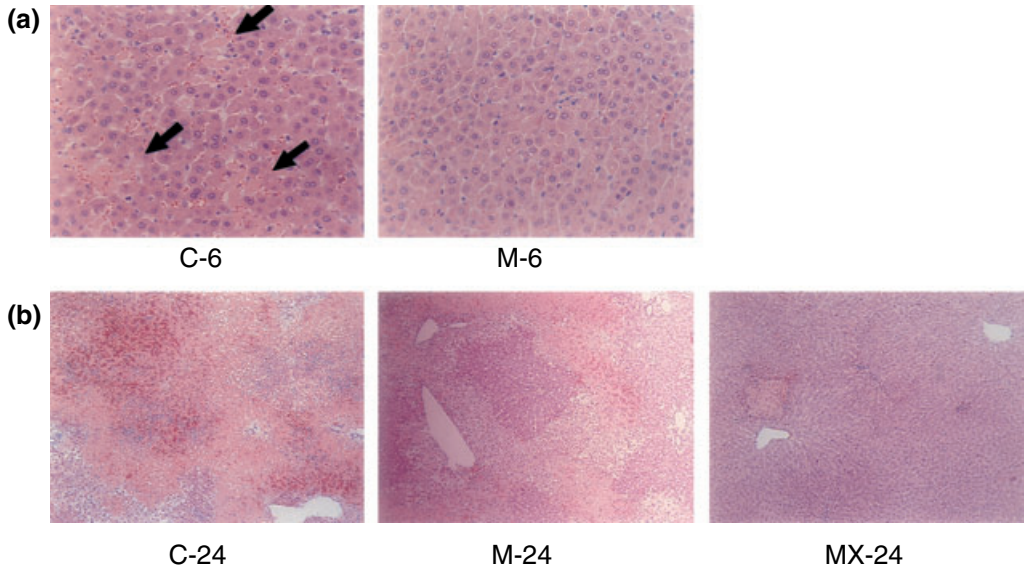

Figure 3 Representative histologic findings in the excised livers at 6 h and 24 h after reperfusion. (a) At 6 h after reperfusion, focal necrosis (arrows) and sinusoidal congestion were observed in the control group (group C-6) but not in the MCI-186-treated group (group M-6). Original magnification ×400. (b) At 24 h after reperfusion, spotty necrosis, ballooning of parenchymal cells, and more severe sinusoidal congestion were seen in the control group (group C-24) and also in the MCI-186-treated group (group M-24) but not in group MX-24 (original magnification ×100).

Table 2. Numerical degree of histologic damage according to the criteria advocated by Suzuki *et al*. (28). Congestion, vacuolization, and necrosis in liver tissue were estimated.

Groups	Congestion	Vacuolization	Necrosis
C-6	3.67 ± 0.21	0.67 ± 0.21	3.33 ± 0.21
M-6	2.33 ± 0.33*	0.50 ± 0.22	2.33 ± 0.21*
C-24	3.17 ± 0.17	3.50 ± 0.22	3.83 ± 0.17
M-24	2.83 ± 0.17	2.33 ± 0.21*	3.67 ± 0.21
MX-24	1.67 ± 0.21*,**	1.83 ± 0.31*	2.50 ± 0.22*,**

*$P < 0.05$ compared with group C.
**$P < 0.05$ compared with group M-24.

C-6, but those of the mRNAs for chemokines CINC-2, MIP-2, MCP-1 and MIP-1α were significantly decreased in group M-6. Expression of MIP-1β mRNA was not suppressed by MCI-186 at any time point. Figure 6c shows the expression of cytokine and chemokine mRNAs at 24 h. Expression was still high in group C-24, and CINC-2 and MIP-2 mRNA expression was suppressed significantly in group M-24. However, no suppression of IL-1, MCP-1, MIP-1α, and MIP-1β mRNA expression was evident. In group MX-24, the expression levels of mRNAs for all cytokines and chemokines were low. The differences in the expression levels of IL-1, MCP-1, MIP-1α and MIP-1β mRNAs were significant in comparison with groups C-24 and M-24.

We also investigated ICAM-1 mRNA expression at 6 h, as shown in Fig. 7. ICAM-1 mRNA expression was high 6 h after reperfusion (group C-6) and gradually decreased thereafter. The expression was significantly reduced in group M-6.

Discussion

We have investigated the usefulness of MCI-186 for the prevention of hepatic warm IRI in a rat model. The results demonstrate that administration of MCI-186 before reperfusion suppressed IRI in the acute phase, but did not result in sufficient suppression in the subacute phase. Additional administration of MCI-186 12 h after reperfusion also suppressed IRI in the subacute phase. This additional administration is presumably necessary because of the short half-life of MCI-186 and the multistep nature of hepatic IRI [1–9].

Free radicals are one of the important factors responsible for hepatic IRI [2–5,29], and several investigators have reported that free radical scavengers attenuate hepatic IRI [3–5,30–35]. Nevertheless, no such scavenger has been used clinically to date recently. MCI-186 is now used clinically and is beneficial after cerebral infarction. There have been several reports describing beneficial effect of MCI-186 on hepatic IRI, e.g. it attenuates liver injury at acute phase [22], it protects against mitochondrial injury [23] and it ameliorates cold IRI [20] or warm IRI [21] in isolated liver perfusion model using Krebs–Henseleit solution. However, none of these studies have focused on the effects of this reagent against various chemical

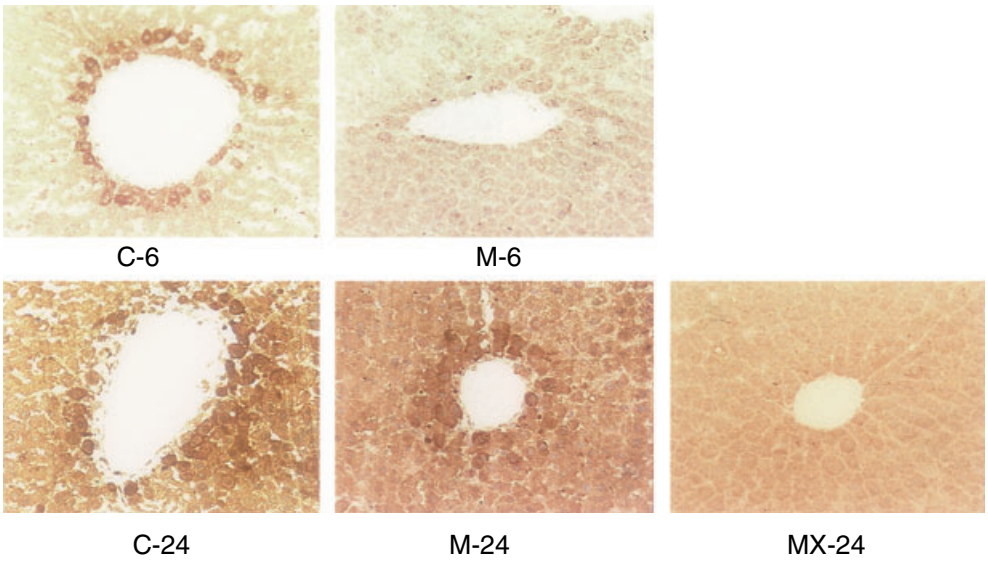

Figure 4 Representative findings of lipid peroxidation immunostaining with monoclonal antibody against 4-hydroxy-2-nonenal (4-HNE) modified proteins in rat livers at 6 h and 24 h after reperfusion. In groups C-6 and C-24, 4-HNE-positive cells were observed. Although 4-HNE staining was faint in group M-6, the number of 4-HNE-positive cells in group M-24 was comparable with that in group C-24. The number of 4-HNE-positive cells was decreased in group MX-24, compared with groups C-24 and M-24 (original magnification ×400).

mediators such as free radicals, cytokines and chemokines, inflammatory cells, adhesion molecules, and also against histologic damage in hepatic IRI model *in vivo*.

The ODFRs activate monocytes (resident or free) and induce nuclear factor κB in hepatic IRI, causing hepatic injury in the early phase [8,12,31,36–38]. TNF-α and IL-1β are potent proinflammatory cytokines secreted mainly by Kupffer cells during hepatic IRI [2–6,8,39,40]. These cytokines induce IL-8 and CINC-2 synthesis [4,41] and upregulate the expression of adhesion molecules such as Mac-1 or ICAM-1 [3,4]. TNF-α also induces chemokines such as epithelial neutrophil-activating protein 78, which plays an important role in neutrophil chemotaxis and activation, and stimulates ODFR production by Kupffer cells [2,4,7,42,43]. IL-1β induces Kupffer cells to produce TNF-α, upregulates ODFR production by neutrophils [4], and also upregulates the expression of nuclear factor κB and CXC chemokines [8]. In this study, the expression of TNF-α and IL-1β was elevated in the early phase after reperfusion, and attenuated in the MCI-186-treated groups.

MCP-1, MIP-1α and MIP-1β are CC chemokines, which exert a chemotactic effect on monocytes and T cells [10–13]. The expression levels of MCP-1 and MIP-1α were reduced in the MCI-186-treated group 3 h and 6 h (acute phase) after reperfusion. MCI-186 reduced monocyte infiltration into the liver, and this might have resulted from suppressed expression of CC chemokines. In the early phase of hepatic IRI, ODFR-stimulated Kupffer cells release MCP-1 [12], upregulates ICAM-1 expression on endothelial cells *in vitro* [44]. Up-regulation of ICAM-1 is one of the important factors involved in the pathogenesis of neutrophil-induced hepatic IRI [45]. These correlations appear to be supported by the present *in vivo* data indicating that suppression of MCP-1 and ICAM-1 expression in the MCI-186-treated groups led to a decrease in monocyte and neutrophil infiltration.

Similar mRNA expression patterns were also observed for members of the CXC chemokine superfamily (CINC-2 and MIP-2). CXC chemokines have potent chemotactic effects on neutrophils. Kupffer cells produce CINC when stimulated with ODFR generated by hypoxanthine and xanthine oxidase [2,3,41], while CINC-2 production can be reduced with a calcium channel blocker [41]. The level of CINC-2 is increased for several hours after reperfusion in rat liver IRI models [2,41]. MIP-2 is also upregulated in early hepatic or renal IRI and has an important role in neutrophil recruitment and organ injury [7,9,13]. Our results indicate that CINC-2 and MIP-2 levels were elevated in the early phase of IRI in the control groups, and we suspect a correlation between the expression of CXC chemokines and neutrophil infiltration. This inference is supported by the observation that both CXC chemokine expression and neutrophil infiltration were suppressed in the MCI-186-treated groups.

In our study, single administration of MCI-186 did not have attenuated infiltration of neutrophils or macrophages in the subacute phase presumably because of its short

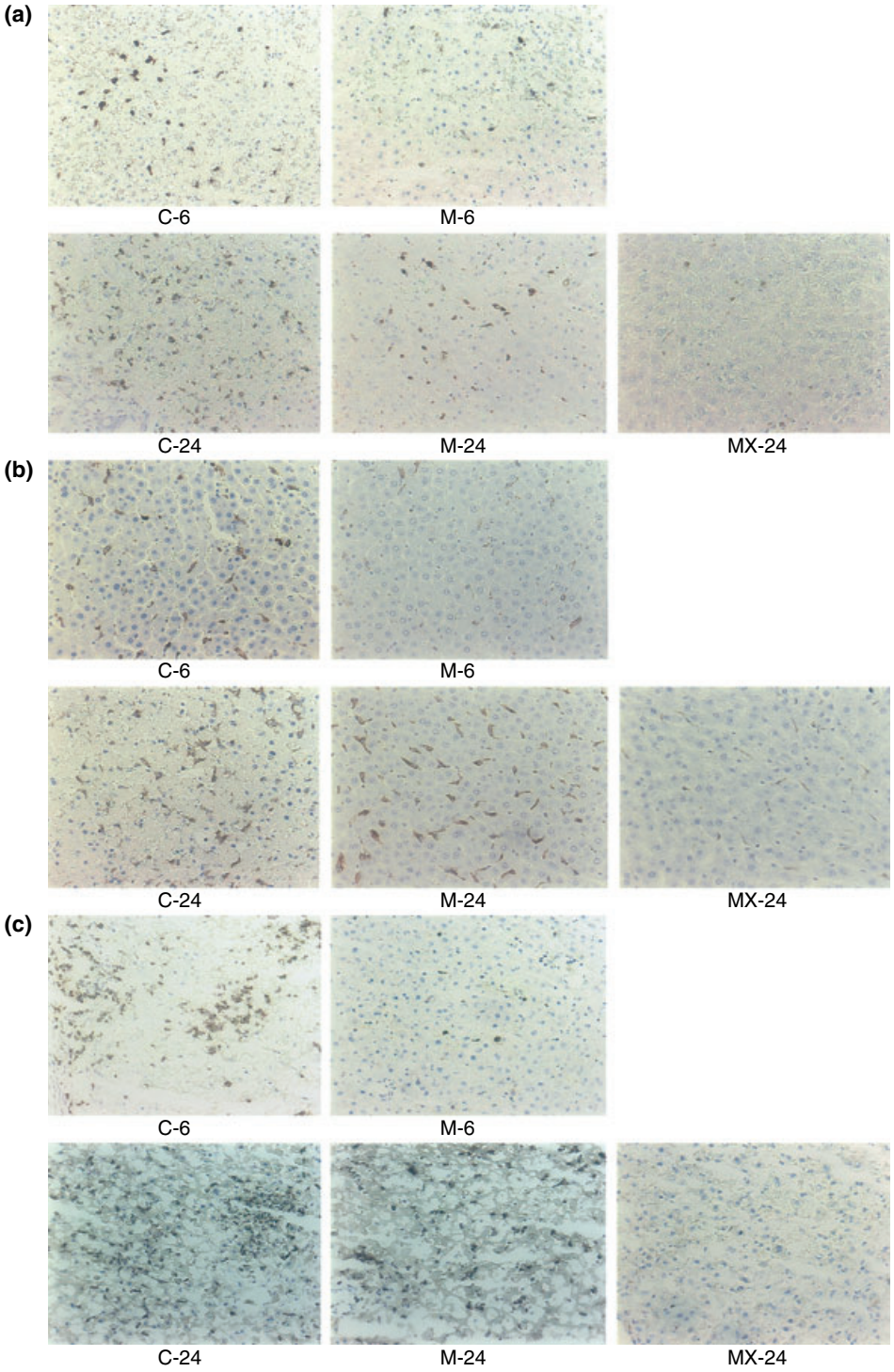

Figure 5 Immunohistochemistry for inflammatory cells in the liver tissues. (a) ED-1-positive cells (resident macrophages). (b) ED-2-positive cells (free macrophages). (c) Neutrophils. The numbers of ED-1- and ED-2-positive cells in the ischemic lobes were increased in group C-6 but absent in group M-6. In group M-24, the numbers of ED-1- and ED-2-positive cells in the ischemic lobes were increased. In group MX-24, the number of macrophages was markedly reduced. Infiltration of neutrophils into the sinusoids was observed in groups C-6 and C-24. Infiltration was suppressed in group M-6, but not in group M-24. In group MX-24, infiltration of neutrophils into sinusoids was demonstrably reduced (original magnification ×400).

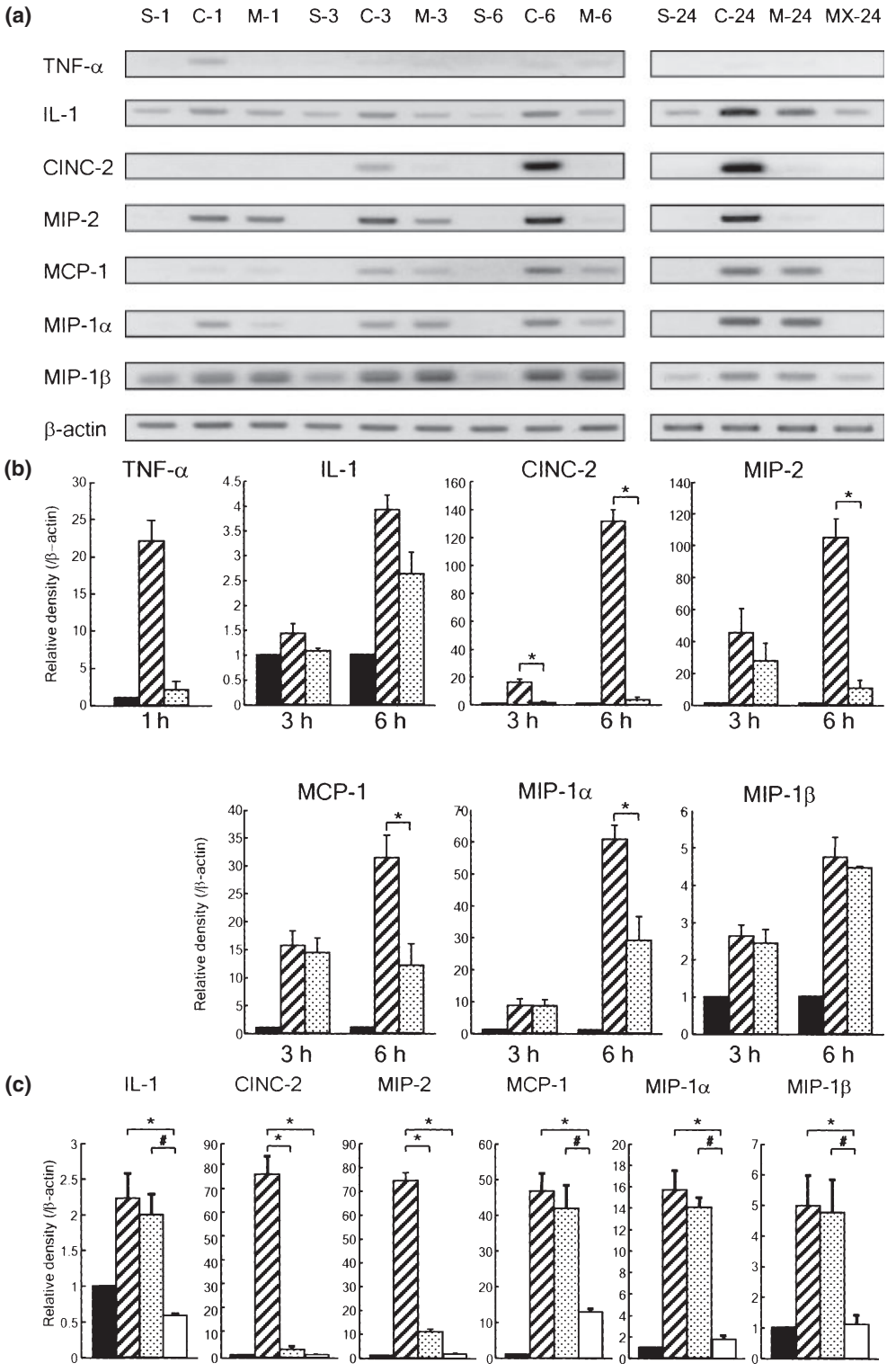

Figure 6 (a) Expression of cytokine and chemokine mRNAs in ischemic lobes of rats. (b) Semiquantification of cytokines and chemokines in the acute phase. (c) Semiquantification of cytokine and chemokines in the subacute phase. Analysis of bands for β-actin and cytokines or chemokines is shown, and the data represent the ratio from five different animals. The expression of cytokines and chemokines was high in group C, but attenuated in group M and group MX. Data represent mean ± SEM. *$P < 0.05$ compared with group C; #$P < 0.05$ compared with group M-24. ■, group S; ▨, group C; ▩, group M; □, group MX.

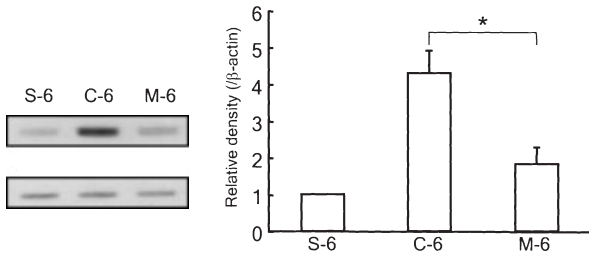

Figure 7 Level of expression of intercellular adhesion molecule (ICAM)-1 mRNA in the ischemic lobe at 6 h after reperfusion. Analysis of bands for ICAM-1 and β-actin is shown, and the data represent the ratio from five different animals. The expression of ICAM-1 was high in group C, but attenuated in group M. Data represent mean ± SEM. $*P < 0.05$ compared with group C.

half-life and the timing of injection [the half-life of MCI-186 is 0.17 h ($t_{1/2}\alpha$) and 0.81–0.85 h ($t_{1/2}\beta$) at a dose of 1.5 mg/kg in clinical laboratory tests] [27]. These pharmacokinetic data suggested the need for a second experiment that included additional administration of MCI-186 (group MX-24). As a result, additional administration of MCI-186 at 12 h after reperfusion suppressed IRI with attenuation of ODFRs and other mediators in the subacute phase.

In summary, MCI-186 attenuates liver injury *in vivo* in a rat warm IRI model, suggesting that this clinically applicable free radical scavenger has the potential to attenuate liver dysfunction in patients after hepatic resection and liver transplantation.

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