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Human Amnion-Derived Mesenchymal Stem Cell Transplantation Ameliorates Liver Fibrosis in Rats

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Background. Mesenchymal stem cells (MSCs) are a valuable cell source in regenerative medicine. Recently, several studies have shown that MSCs can be easily isolated from human amnion. In this study, we investigated the therapeutic effect of transplantation of human amnion-derived MSCs (hAMSCs) in rats with liver fibrosis. **Methods.** Liver fibrosis was induced by an intraperitoneal injection of 2 mL/kg of 50% carbon tetrachloride twice a week for 6 weeks. At 3 weeks, hAMSCs (1×10^6 cells) were transplanted intravenously. Rats were sacrificed at 7 weeks, and histological analyses and quantitative reverse-transcription polymerase chain reaction were performed. In vitro experiments were conducted to investigate the effect of hAMSCs on the activation of Kupffer cells. **Results.** Transplantation of hAMSCs significantly reduced the fibrotic area, deposition of type-I collagen, the number of α -smooth muscle actin–positive hepatic stellate cells, and CD68-positive Kupffer cells in the livers. messenger RNA expression of α -smooth muscle actin and tissue inhibitor of metalloproteinase-1 was significantly decreased and the expression of matrix metalloproteinase-9 and hepatocyte growth factor was significantly increased in the liver of hAMSC-treated rats. Transplantation of hAMSCs at 3 weeks plus 5 weeks did not have an additive effect. In vitro experiments demonstrated that Kupffer cell activation induced by lipopolysaccharide was significantly decreased by culturing with conditioned medium obtained from hAMSCs. **Conclusions.** Transplantation of hAMSCs provided significant improvement in a rat model of liver fibrosis, possibly through the inhibition of Kupffer cell and hepatic stellate cell activation. hAMSCs may be a potential new treatment for liver fibrosis.

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Liver cirrhosis is a progressed stage of fibrosis caused by chronic liver injury caused by various factors, such as viral infections, alcohol, drugs, and chemical toxicity.¹ The only effective available treatment for end-stage liver cirrhosis is liver transplantation. However, because of the lack of donors, complications, and organ rejection after liver transplantation, alternative treatments are needed.²

Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into various lineages, including bone, cartilage, or fat, and are present in adult tissues.³ At present, MSCs have been investigated in regenerative medicine because of their ability to differentiate and their potential to heal damaged tissues by the secretion of various growth factors and anti-inflammatory molecules.^{4,5} The efficacy of

autologous and allogeneic MSC transplantation in patients with liver cirrhosis has recently been reported.^{6–9}

The fetal membrane comprises amnion and chorion, which envelops the developing fetus. Although the human fetal membrane is generally discarded as medical waste after delivery, fetal tissues have been found to be rich sources of MSCs.^{10–13} We have previously reported that systemic administration of amnion-derived MSCs (AMSCs) improved rats with hind-limb ischemia,¹⁴ myocarditis,^{15,16} glomerulonephritis,¹⁷ and ischemia-reperfusion–induced acute kidney injury¹⁸ by inducing angiogenesis and anti-inflammatory effects.

In this study, we investigated whether the administration of human AMSCs (hAMSCs) improves carbon tetrachloride (CCl₄)–induced liver fibrosis in rats and explored its underlying mechanisms.

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MATERIALS AND METHODS

Isolation and Expansion of hAMSCs

The Medical Ethical Committee of Hokkaido University Graduate School of Medicine, Sapporo, Japan approved this work, and all pregnant women gave written informed consent. The human fetal membrane was obtained during caesarean deliveries, and the amnion was manually peeled from the chorion. The hAMSCs were isolated and expanded by digestion with collagenase type III (Worthington Biochemical Corporation, Lakewood, NJ), followed by seeding in uncoated plastic dishes with minimal essential medium (MEM) α (Life Technologies, Carlsbad, LA) supplemented with 10% fetal bovine serum (Life Technologies), 100 U/mL of penicillin, and 100 ng/mL of streptomycin (Wako Pure Chemical Industries, Osaka, Japan). The culture was maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂. After 3 to 4 days in culture, the nonadherent cells were removed, and the adherent cells were maintained in culture until they reached 80% confluence. The passage was performed using 0.5% trypsin-ethylenediaminetetraacetic acid (Life Technologies).

Differentiation of hAMSCs Into Adipocytes and Osteocytes

The hAMSCs were seeded onto 6-well plates, and differentiation into adipocytes and osteocytes was induced when the hAMSCs were 80% to 90% confluent. To induce differentiation into adipocytes, hAMSCs were cultured with hMSC adipogenic differentiation medium (Lonza, Basel, Switzerland), according to the manufacturer's instructions. After 3 weeks of differentiation, cells were stained with oil red O (Sigma-Aldrich, St. Louis, MO) to confirm differentiation. To induce differentiation into osteocytes, hAMSCs were cultured in hMSC osteogenic differentiation medium (Lonza), according to the manufacturer's instructions. After 2 weeks of differentiation,

cells were stained with Alizarin red S (Sigma-Aldrich) to confirm differentiation.

Flow Cytometry

Cultured hAMSCs were stained using the Human MSC Analysis Kit (BD, Franklin Lakes, NJ), which included fluorescein-isothiocyanate-conjugated antibody against CD90, PerCP-Cy5.5-conjugated antibody against CD105, phycoerythrin-conjugated CD44, and allophycocyanin-conjugated antibody against CD73 as well as a negative cocktail (phycoerythrin-conjugated CD11b, CD19, CD34, CD45, and HLA-DR), according to the manufacturer's instructions. Cells were analyzed by a flow cytometer (FACSCanto II, BD).

Animals

The experimental protocol was approved by the Animal Care and Use Committees of Hokkaido University. Six-week-old male Sprague-Dawley rats were procured from Japan SLC (Hamamatsu, Japan), and 3 rats were housed per cage in a temperature-controlled room (24°C) on a 12-hour/12-hour light/dark cycle. All rats had ad libitum access to standard chow and water.

Induction of Liver Fibrosis and hAMSC Transplantation

Liver fibrosis was induced by an intraperitoneal injection of 2 mL/kg of 50% CCl₄ (Wako Pure Chemical Industries) in olive oil twice a week for 6 weeks. In the Control group, rats were injected with olive oil alone (Figure 1). One million hAMSCs suspended in 200 μ L of phosphate-buffered saline were intravenously injected through the penile vein after 2 weeks of CCl₄ treatment. Two hundred microliters of phosphate-buffered saline was injected in the untreated rats and those treated with CCl₄ (Figure 1).

Histological Examination

All rats were sacrificed after 6 weeks of CCl₄ treatment. The left lobe of the liver was removed, fixed in 40 g/L of

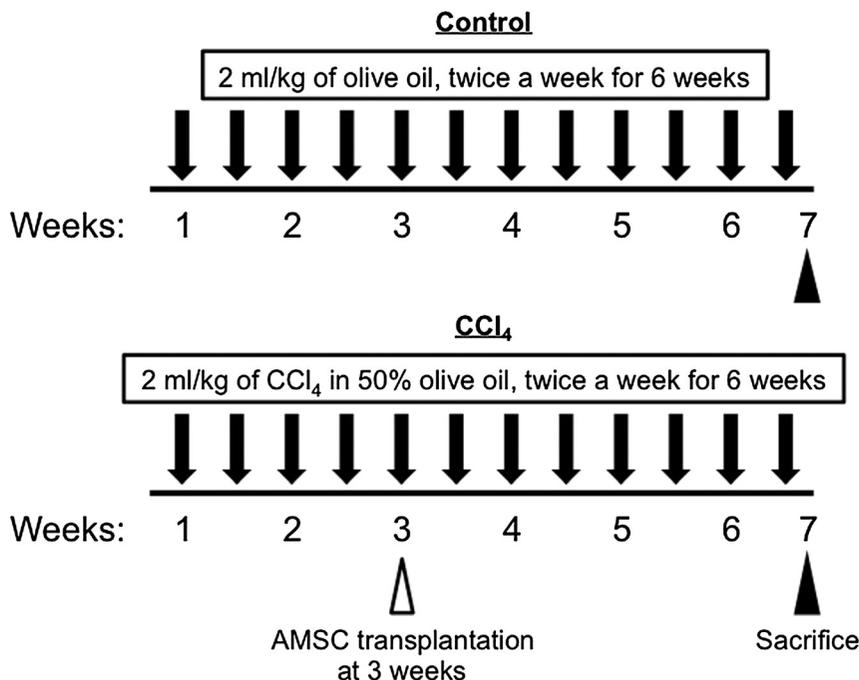


FIGURE 1. Experimental protocol for CCl₄-induced liver fibrosis. Rats received 2 mg/kg of CCl₄ in 50% olive oil twice a week for 6 weeks. hAMSCs (1×10^6 cells) were infused intravenously at 3 weeks. All rats were sacrificed at 7 weeks.

formaldehyde saline, embedded in paraffin, and cut into 5- μ m sections. Tissue sections were stained with Masson trichrome. Ten random fields on a section from each rat were photographed, and blue-stained areas were calculated from the entire liver cross-sectional area (% , $\times 20$) with a digital image analyzer (WinROOF; Mitani Co., Fukui, Japan).

Immunohistochemical Examination

The tissue sections were stained with anti-rat type I collagen antibody (dilution, 1:100,000; LSL, Tokyo, Japan) for 60 minutes at room temperature. To assess the activation of hepatic stellate cells (HSCs), the tissue sections were stained with anti-rat α -smooth muscle actin (SMA) antibody (1:800, Thermo Scientific, Waltham, MA, USA) for 30 minutes at room temperature. To assess the infiltration of Kupffer cells, the tissue sections were stained with anti-rat CD68 monoclonal antibody (dilution, 1:50; AbD Serotec, Kidlington, United Kingdom) for 40 min at room temperature. Ten random fields on a section from each rat were photographed, and stained areas were calculated from the entire liver cross-sectional area.

RNA Isolation and Quantitative Reverse-Transcription Polymerase Chain Reaction

Total RNA of the rat liver was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and 1 μ g of the total RNA was reverse-transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen). Polymerase chain reaction (PCR) amplification was performed using a 25- μ L reaction mixture that contained 1 μ L of cDNA and 12.5 μ L of Platinum SYBR Green PCR Mix (Invitrogen, Carlsbad, CA). β -actin messenger RNA that was amplified from the same samples served as an internal control. After initial denaturation at 95°C for 2 minutes, a 2-step cycle procedure was used (denaturation at 95°C for 15 seconds, annealing and extension at 60°C for 1 minute) for 40 cycles in a 7700 Sequence Detector (Applied Biosystems, Foster City, CA). Gene expression levels were determined using the comparative threshold cycle (ddCt) method with β -actin used as an endogenous control. Data were analyzed with Sequence Detection Systems software (Applied Biosystems). Primer sequences are shown in Table 1.

In Vitro Experiments Using Rat Kupffer Cells

Livers were excised from 8-week-old Sprague–Dawley rats, perfused with collagenase and centrifuged twice (90g for 1 minute). The cell fraction in the supernatant was centrifuged again (690g for 5 minutes), and the obtained cell pellet was suspended with serum-free Dulbecco MEM (DMEM, Life Technologies). The cells were then seeded onto a non-coated plate for 45 minutes at room temperature, and the adhesive cells were regarded as Kupffer cells. The next day, cells were treated with 100 ng/mL lipopolysaccharide (LPS) in standard medium or conditioned medium obtained from hAMSCs for 4 hours. A conditioned medium was collected by culturing subconfluent hAMSCs with serum-free MEM α for 48 hours, and standard medium was collected by incubating serum-free MEM α without hAMSCs for 48 hours. Cell number was evaluated by measuring the cellular level of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, indicative of the mitochondrial function of living cells and cell viability, using a CellTiter96 AQueous One Solution Kit (Promega, Madison, WI) and a microplate reader (490 nm, Promega). Concentration of rat TNF- α in the culture media was measured using the

TABLE 1.
qRT-PCR Primer Sequences

Gene	Primer sequence
α -SMA	F: CGGGCTTTGCTGGTGATG R: CCCTCGATGGATGGGAAA
MMP-2	F: CACCACCCGAGGACTATGACC R: TGTTGCCCGAGAAAGTGAAG
MMP-9	F: TGGAACTCACACAACGCTCTTCA R: TCACCCGGTTGTGGAACTC
TIMP-1	F: TCCTCTTGTGCTATCATTGATAGCT R: CGCTGGTATAAGGTGGTCTCGAT
TGF- β	F: CCCCTGGAAAGGGCTCAACAC R: TCCAACCCAGGTCCTTCTAAAGTC
HGF	F: GACATTCCTCAGTGTTGAGAAGTTG R: TGCCTGATTCTGTGTGATCCA
VEGF	F: CAGCTATTGCCGTCCAATTGA R: CCAGGGCTTCATCATTGCA
EGF	F: CCCGTGTCTTCTCGAGTTCC R: TGTAACCGTGGCTTCCTCT
TGF- α	F: CATCACTGCCCTGGTGGTAGT R: GGACCTGACAGCAGTGGATCA
IL-10	F: ATAAAAGCAAGGAGTGGAG R: TGGAGAGAGGTACAAACGAG
β -actin	F: CCAACCGTGAAGATGACC R: ACCAGAGGCATACAGGGACA

F, forward primer; R, reverse primer.

Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), following the manufacturer's instructions.

Statistical Analysis

Data are shown as mean \pm SEM. Parameters among the groups were compared by 1-way analysis of variance, followed by a Tukey test. Differences were considered significant at *P* less than 0.05.

RESULTS

Characterization of hAMSCs

To evaluate the multipotency of hAMSCs, we induced differentiation of cultured hAMSCs into adipocytes and osteocytes. The hAMSCs differentiated into adipocytes and osteocytes, as demonstrated by oil red O and Alizarin red S staining, respectively (Figure 2A). Flow cytometry of cultured hAMSCs showed that they expressed CD44, CD73, CD90, and CD105, but not CD11b, CD19, CD34, CD45 or HLA-DR, which is a characteristic expression pattern for MSCs (Figure 2B).¹⁹

Effect of hAMSC Transplantation on Histological Parameters in CCl₄-Treated Rats

We started with 6 rats for Control group, and 15 rats for CCl₄ group and CCl₄ + AMSC group, respectively. In the CCl₄ group, 3 rats died at week 6. In the CCl₄ + AMSC group, 2 rats died at week 6, and 3 rats died at the day of AMSC transplantation due to the overdose of pentobarbital. Therefore, we analyzed 6 control rats, 12 CCl₄ rats, and 10 CCl₄ + AMSC rats. In the CCl₄ group, severe fibrosis was observed; however, the fibrosis was significantly attenuated by hAMSC transplantation at week 7 (Figure 3A). Consistent with this finding, the upregulated expression of type I collagen by CCl₄ treatment was also attenuated by hAMSC

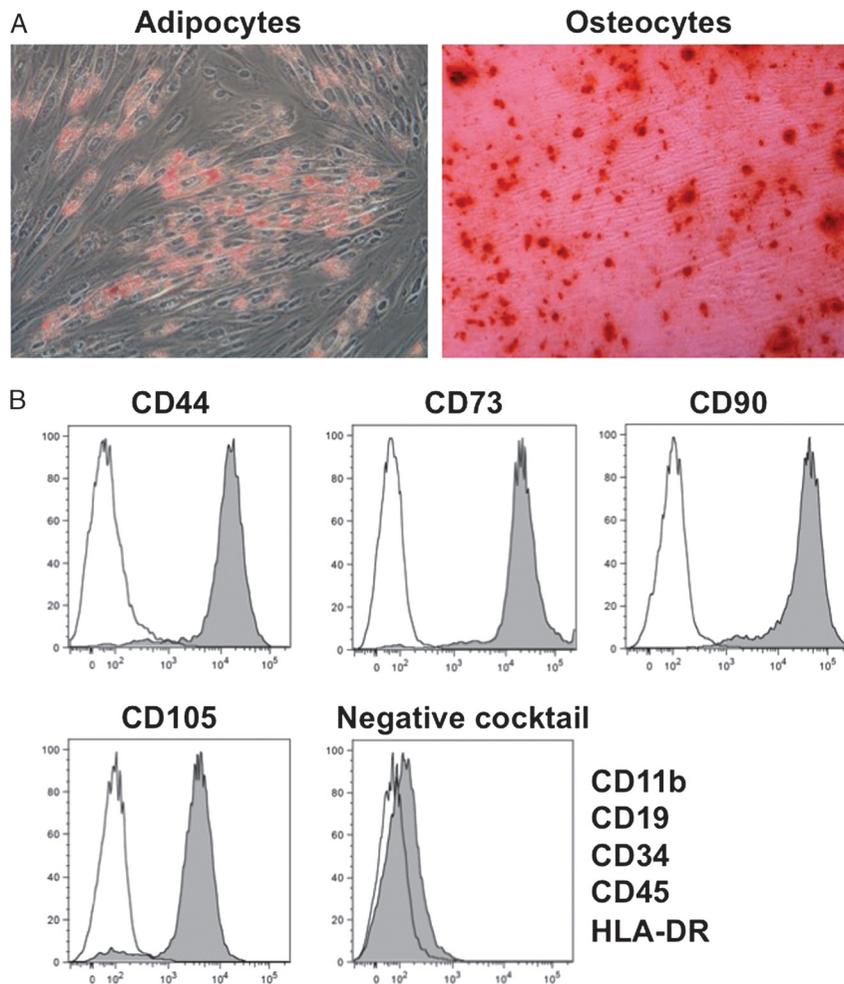


FIGURE 2. Characterization of cultured hAMSCs. A, Multipotency of hAMSCs. Differentiation into adipocytes was confirmed by the existence of lipid vesicles stained with oil red O (left). Differentiation into osteocytes was confirmed by the existence of mineral nodule deposition stained with Alizarin red S (right). B, Flow cytometry of hAMSCs. The negative cocktail contained antibodies against CD11b, CD19, CD34, CD45, and HLA-DR. Closed areas indicate staining with a specific antibody, whereas open areas represent staining with isotype control antibodies.

transplantation (Figure 3B). The expression of α -SMA, a marker for the activation of HSCs, was significantly increased in the CCl₄ group; however, hAMSC transplantation significantly suppressed this increase (Figure 3C). The expression of CD68, a marker for Kupffer cells, was significantly increased in the CCl₄ group; however, hAMSC transplantation significantly decreased the infiltration of CD68-positive Kupffer cells (Figure 3D).

Effects of hAMSC Transplantation on Gene Expression in CCl₄-Treated Rats

We next examined the expression profile of fibrosis-related genes in the liver. The CCl₄ treatment significantly increased the expression of α -SMA, matrix metalloproteinase (MMP)-2, tissue inhibitor of metalloproteinase (TIMP)-1, and TGF- β (Figure 4A, B, D, and E), and the expressions of α -SMA and TIMP-1 were significantly decreased by hAMSC transplantation (Figure 4A and D). The expressions of MMP-9 and hepatocyte growth factor (HGF) were not affected by CCl₄ treatment, but significantly increased by hAMSC transplantation (Figure 4C and F). The expressions of VEGF and TGF- α were significantly decreased by CCl₄ treatment, but these decreases were not attenuated by

hAMSC transplantation (Figure 4G and I). The expressions of EGF and IL-10 were not affected by CCl₄ treatment and hAMSC transplantation (Figure 4H and J), respectively.

Effect of Biweekly Transplantation of hAMSCs on Histological Parameters in CCl₄-Treated Rats

To evaluate the effect of biweekly transplantation of hAMSCs, hAMSCs were transplanted after 2 and 4 weeks of CCl₄ treatment, and evaluated at 7 weeks. We started with 6 rats for Control group, and 13 rats for CCl₄ group, CCl₄ + AMSC1 group, and CCl₄ + AMSC2 group, respectively. In the CCl₄ group, 2 rats died at weeks 2 and 7, and 2 rats died at week 3 due to the overdose of pentobarbital. In the CCl₄ + AMSC1 group, 1 rat died at week 6, and 3 rats died at the day of AMSC transplantation due to the overdose of pentobarbital. In the CCl₄ + AMSC2 group, 1 rat died at week 7, and 1 rat died at the day of AMSC transplantation due to the overdose of pentobarbital. Therefore, we analyzed 6 control rats, 9 CCl₄ rats, 9 CCl₄ + AMSC1 rats, and 11 CCl₄ + AMSC2 rats. Histological examination demonstrated that there is no additive affect by double injection of hAMSCs (Figure 5A-D).

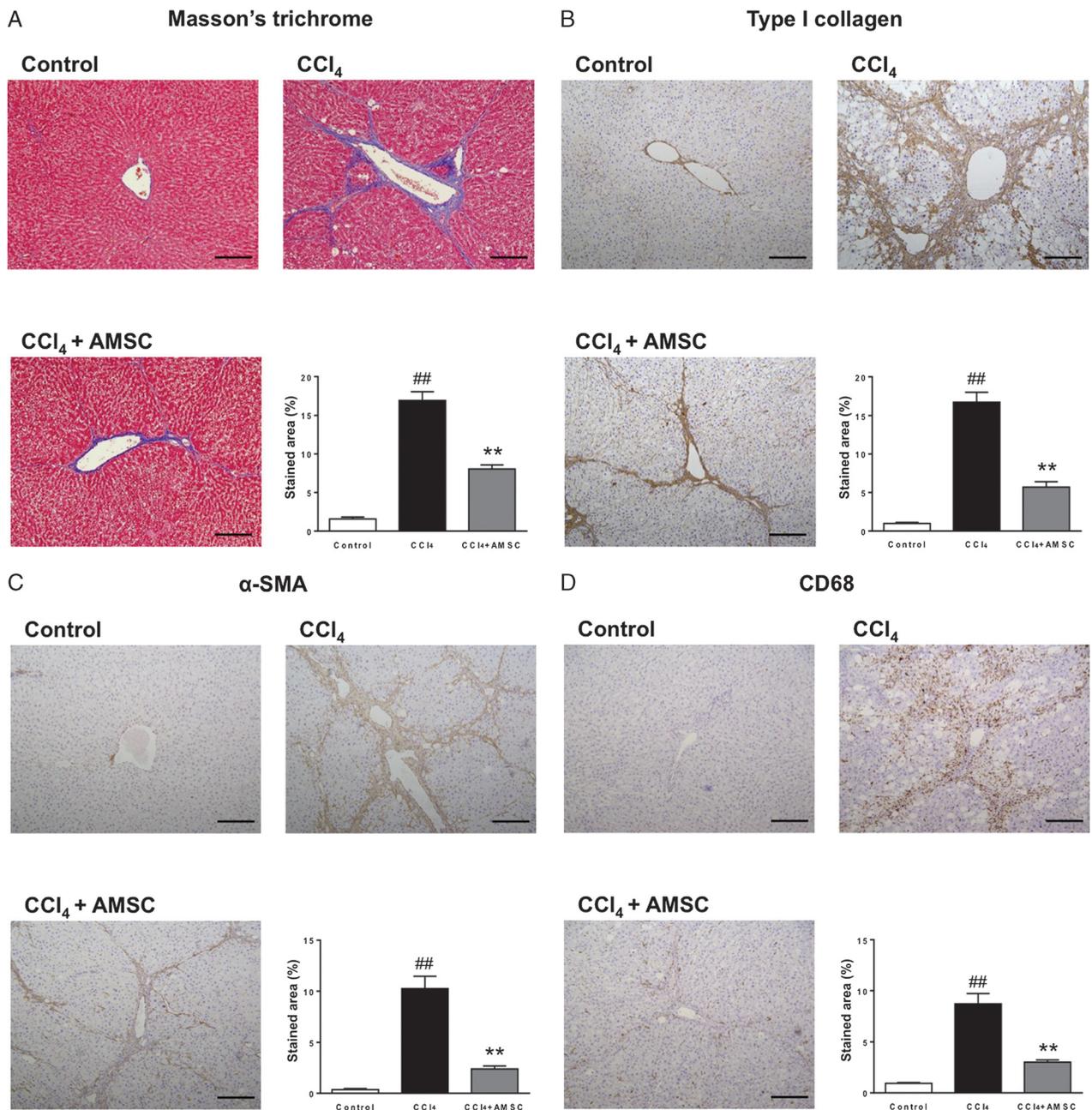


FIGURE 3. Effect of hAMSC transplantation on histological parameters in CCl₄-treated rats (A) Masson's trichrome staining. Fibrotic area was stained blue and calculated from the entire liver cross-sectional area. Immunohistochemical analyses of (B) Type I collagen, (C) α-SMA and (D) CD68 were performed, and the stained areas were calculated from the entire liver cross-sectional area. Scale bars, 200 μm. The values are reported as the mean ± SEM (n = 6 in control group, n = 12 in CCl₄ group, and n = 10 in CCl₄ + AMSC group). ##P < 0.01 versus Control group. **P < 0.01 versus CCl₄ group.

Effect of hAMSC-Conditioned Medium on the Activation of Kupffer Cells In Vitro

To investigate the effect of hAMSC on Kupffer cell activation, Kupffer cells were isolated from the rat liver, stimulated with LPS, and cultured with conditioned medium obtained from hAMSC culture. The LPS treatment did not affect the cell number (Figure 6A), but markedly increased the secretion of TNF-α. Interestingly, exposure to hAMSC-conditioned medium significantly suppressed the activation of isolated Kupffer cells. This finding was consistent across hAMSC conditioned medium obtained from 3 different donors (Figure 6B).

DISCUSSION

In this study, we investigated the therapeutic potential of hAMSCs in rats with liver fibrosis and found that (1) hAMSC transplantation ameliorated liver fibrosis; (2) hAMSC transplantation suppressed the activation of HSCs; (3) hAMSC transplantation suppressed the infiltration of Kupffer cells; and (4) hAMSC-conditioned medium downregulated the activation of Kupffer cells.

The therapeutic effect of MSCs derived from the bone marrow, adipose tissue, and umbilical cord has been recently reported in a liver fibrosis model.²⁰⁻³⁵ Although each experimental protocol is different in terms of the duration of CCl₄

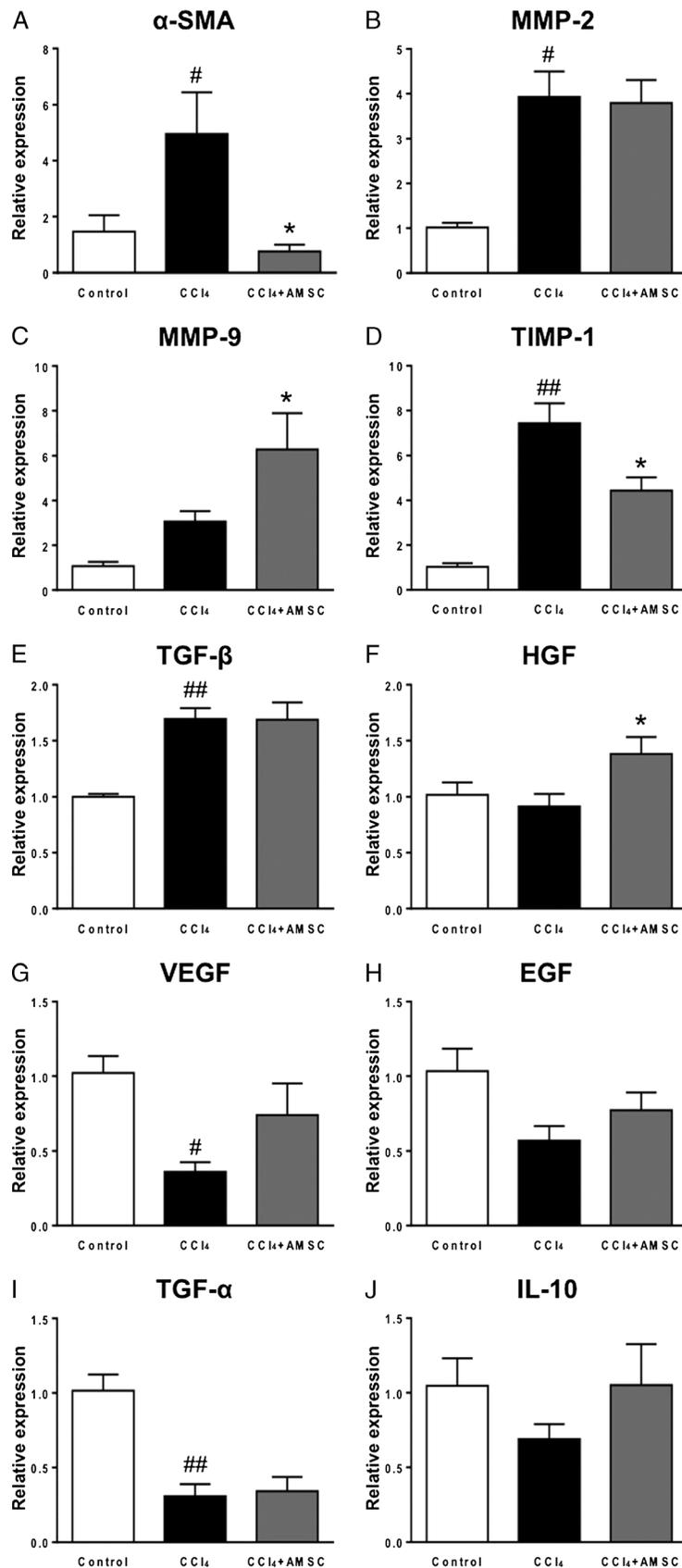


FIGURE 4. Gene expression analysis of hAMSC transplantation in CCl₄-induced liver fibrosis, qRT-PCR for (A) SMA, (B) MMP-2, (C) MMP-9, (D) TIMP-1, (E) transforming growth factor (TGF)-β, (F) HGF, (G) vascular endothelial growth factor (VEGF), (H) epidermal growth factor (EGF), (I) TGF-α, and (J) IL-10. The values are reported as the mean ± SEM of (n = 6 in control group, n = 12 in CCl₄ group, and n = 10 in CCl₄ + AMSC group). #P < 0.05, ##P < 0.01 versus Control group. *P < 0.05 versus CCl₄ group. qRT-PCR indicates quantitative reverse transcription-polymerase chain reaction.

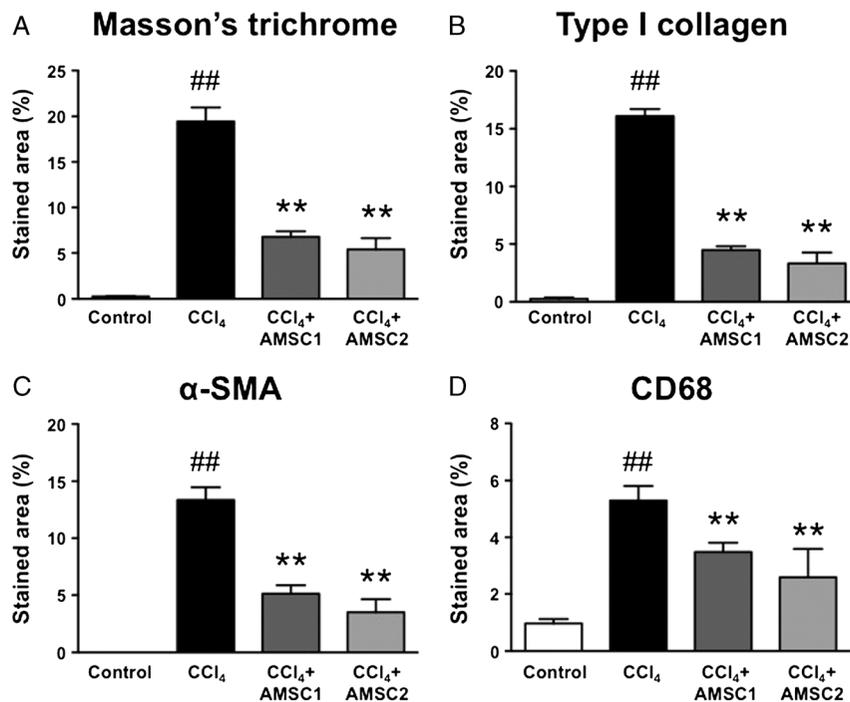


FIGURE 5. Effect of biweekly transplantation of hAMSCs on histological parameters in CCl₄-treated rats, (A) Masson trichrome staining. Fibrotic area was stained blue, and calculated from the entire liver cross-sectional area. Immunohistochemical analyses of (B) type I collagen, (C) SMA, and (D) CD68 were performed, and the stained areas were calculated from the entire liver cross-sectional area. The values were the mean \pm SEM ($n = 6$ in control group, $n = 9$ in CCl₄ group, $n = 9$ in CCl₄ + AMSC1 group, and $n = 11$ in CCl₄ + AMSC2 group). ## $P < 0.01$ versus Control group. ** $P < 0.01$ versus CCl₄ group.

administration (4 to 12 weeks) and the number of MSCs transplanted (5×10^5 to 3×10^7 cells/rat), it appeared that MSCs suppressed the fibrosis of liver in most reports. In the present study, liver fibrosis was induced by the administration of CCl₄ for 6 weeks, and hAMSCs (1×10^6 cells/rat) were transplanted at 3 weeks. Recently, the use of hAMSCs in a liver fibrosis in mice has been reported.³⁶ This group showed that hAMSC transplantation suppressed the activation of HSCs, decreased hepatocyte apoptosis, and promoted liver regeneration. Accordingly, our study showed that hAMSCs suppressed the activation of HSCs as demonstrated by immunohistochemical examination and quantitative reverse transcription-PCR. In addition, we demonstrated that hAMSC transplantation suppressed the infiltration of Kupffer cells. Furthermore, *in vitro* experiments demonstrated that hAMSC-conditioned medium suppressed the activation of Kupffer cells, which are known to modulate inflammation in the development of liver fibrosis.³⁷ Various studies have shown that Kupffer cells act as an integral factor in hepatocyte apoptosis, inflammation, and fibrosis.³⁸⁻⁴⁰ In addition, we have very recently demonstrated that AMSC-conditioned medium suppressed the nuclear translocation of NF- κ B, but not the phosphorylation of I κ B in macrophages.⁴¹ Therefore, the anti-inflammatory effect of AMSCs to Kupffer cells/macrophages could contribute to the anti-fibrotic effect. Conversely, HSCs have been identified as an important cellular source of extracellular matrix (ECM) in liver fibrosis.⁴² Activated HSCs undergo a phenotypic trans-differentiation to myofibroblasts expressing α -SMA and produce a numerous ECM molecules, such as collagen.⁴³ In this study, we investigated the effect of hAMSC transplantation on HSCs in CCl₄-treated rats, which showed that hAMSCs

decreased HSC activation, as indicated by the decreased expression of α -SMA.

The TIMP-1 expression has been shown to be increased in the development of liver fibrosis both in murine models and human samples.⁴⁴ In addition, it has been demonstrated that TIMP-1 significantly attenuated spontaneous resolution of liver fibrosis by the combination of a net reduction of MMP activity and suppression of apoptosis in HSCs.⁴⁵ We found that the expression of TIMP-1 was significantly decreased and MMP-9 was further increased by hAMSC transplantation. The other possible explanation for the fibrotic resolution is hepatic regeneration. The HGF plays an essential part in the development and regeneration of the liver and shows anti-apoptotic activity in hepatocytes.⁴⁶ We found that the expression of HGF was significantly increased by hAMSC transplantation. Recently, it has been reported that IL-10 production by infused bone marrow cells is a key negative regulator of the liver fibrosis.⁴⁷ However, the expression of IL-10 was not significantly increased by hAMSC transplantation in our study. Therefore, hAMSC transplantation may ameliorate liver fibrosis not only by suppressing the infiltration of Kupffer cells and HSC activation but also by inducing MMP-9, inhibition of Timp1 as well as induction of HGF. However, because the upregulation of HGF expression by hAMSC transplantation was relatively small, contribution of HGF induction in the improvement of liver fibrosis might be small.

In this study, we investigated the efficacy of biweekly transplantation to see if there was an additive effect. In this model, there is no additive effect by double injection of hAMSCs. Considering that liver fibrosis in this model progresses over the course of weeks, it is possible that hAMSC transplantation

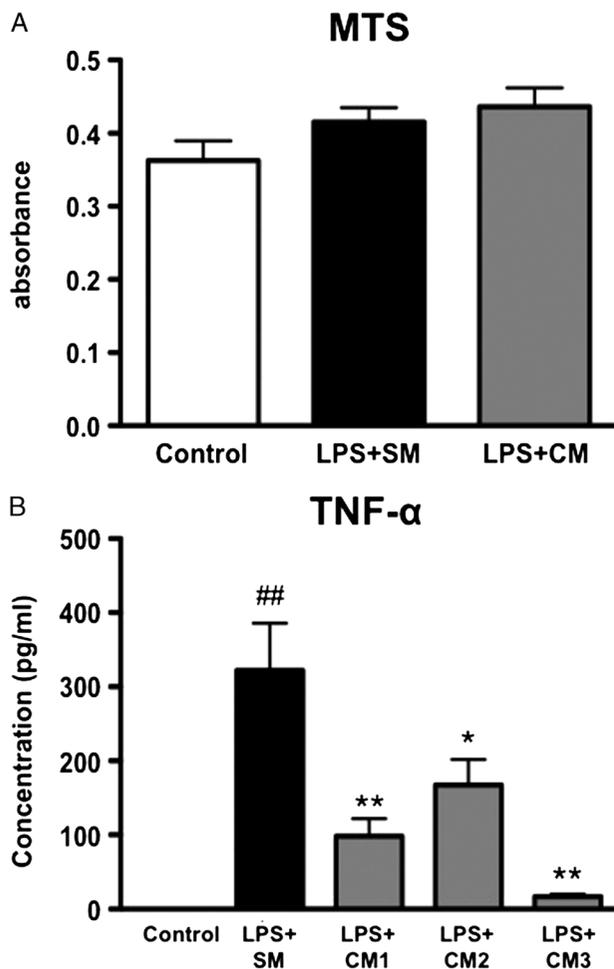


FIGURE 6. Effect of hAMSC-CM on the activation of Kupffer cells in vitro. (A) Primary Kupffer cells were treated with 100 ng/ml of LPS in standard medium (SM) or hAMSC-CM for 4 h. The number of cells was evaluated by MTS assay. (B) Primary Kupffer cells were treated with 100 ng/ml of LPS in standard medium (SM) or hAMSC-CM for 4 h. CM was obtained from hAMSCs of 3 different donors (CM1, CM2, and CM3). Secretion of TNF- α from the Kupffer cells was measured by ELISA. The values were the mean \pm SEM. ## P < 0.01 versus Control. * P < 0.05, ** P < 0.01 versus LPS + SM. ELISA indicates enzyme-linked immunosorbent assay; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; CM, conditioned medium.

every couple of months would be sufficient in the clinical setting. A future clinical trial should investigate the interval of hAMSC transplantation for the treatment of liver fibrosis.

Recently, several clinical studies using human MSCs in liver fibrosis have been reported.^{8,9,48-51} Autologous bone marrow MSC infusion therapy has been reported to be safe and effective for patients with liver failure caused by hepatitis B⁴⁹ and hepatitis C.⁵⁰ They showed that MSC transplantation contributed to the improvement in serum albumin levels, total bilirubin, prothrombin time, and model for end-stage liver disease score⁴⁹ or in the Child score, model for end-stage liver disease score, fatigue score, and performance status⁵⁰ without severe adverse effects. In addition, umbilical cord-derived MSC therapy has been reported to improve serum albumin levels and total serum bilirubin levels and to reduce ascites in patients with decompensated liver cirrhosis.⁸ Other clinical trials using human MSCs in liver fibrosis are being conducted in several countries (<http://www.clinicaltrials.gov>). Recent reports have

suggested similar efficacy of AMSCs for several other diseases.⁵²⁻⁵⁴ It has been demonstrated that intravenous infusion of hAMSCs ameliorates inflammation and fibrosis in the lung induced by bleomycin in mice.⁵⁴ They tested the efficacy of AMSCs, bone marrow MSCs, and human amniotic epithelial cells, and concluded that AMSC transplantation was more effective in reducing lung injury. In addition, very recently, a first-in-human pilot study using fetal membrane-derived MSCs has been conducted to treat nine patients with steroid-refractory acute graft-versus-host disease. The fetal membrane-derived MSCs appeared safe for intravenous infusion to most patients, and the overall response rate in severe refractory acute graft-versus-host disease appeared to be similar to the rate observed while using bone marrow-derived MSCs.⁵³ This is encouraging because it is rather invasive to aspirate bone marrow from donors. It is non-invasive to obtain amnion because it is discarded as a medical waste, and AMSCs are originated from fetus, but not mother. In addition, we can use younger cells than MSCs from other tissues, such as bone marrow or adipose tissue. In addition, we have recently demonstrated that AMSCs produce a large amount of prostaglandin E2, one of the key modulators of inflammation.⁵⁵ Therefore, AMSC transplantation would be more pertinent for patients with liver fibrosis that may require repeated cell therapy for a long time.

In conclusion, human AMSC transplantation ameliorated fibrosis in a rat model of liver fibrosis, possibly through the suppression of Kupffer cell infiltration and activity of HSCs. Considering that the fetal membrane is generally discarded as medical waste and can be obtained without an invasive procedure, AMSC transplantation should be considered as a therapeutic strategy for the treatment of liver cirrhosis.

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