

Intrahepatic transplantation of adipose-derived stem cells attenuates the progression of non-alcoholic fatty liver disease in rats

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Abstract. Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of chronic liver injury affecting the general health of various populations. In the present study, adipose tissue-derived stem cells (ADSCs), which were isolated from the adipose tissues of Sprague-Dawley rats, were transplanted into the liver of high-fat-diet-induced NAFLD rats via the portal vein to attenuate the disease progression of NAFLD. The results demonstrated that ADSC transplantation reduced the serum levels of alanine aminotransferase, total bilirubin, total cholesterol, triglycerides and fatty acids, and reduced the content of malondialdehyde in the liver homogenates. By contrast, ADSC transplantation caused a significant increase in superoxide dismutase activity. These data suggested that the ADSC transplantation improved the liver function, and reduced lipid metabolism and oxidative stress. In addition, the hepatic pathological changes were decelerated, lipid accumulation was reduced, and serum levels of the pro-inflammatory factors, tumor necrosis factor- α and interleukin-6, were down-regulated by the ADSC transplantation. Taken together, transplantation with ADSCs attenuates the disease progression of high-fat-diet induced NAFLD, therefore, may offer a potential therapeutic approach for the treatment of NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of chronic liver injury, which affects 10-24% of the general population worldwide (1). The prevalence of NAFLD has markedly increased, which is associated with rates of severe obesity, insulin resistance and other metabolic syndromes (2). The underlying pathogenesis of NAFLD remains to be fully elucidated, however, the pathological changes may be explained by the 'two-hit hypothesis' (3). The 'first hit' consists of the accumulation of hepatic lipids, which is predominantly associated with insulin resistance, while the 'second hit' consists of oxidative stress, mitochondrial dysfunction and inflammation, which may aggravate the existing steatosis and lead to non-alcoholic steatosis hepatitis (NASH) (1,4). At present, there remains no satisfactory therapeutic treatment for NAFLD.

Mesenchymal stem cells (MSCs) possess the ability to differentiate into multiple cells, including adipocytes, osteocytes, hepatocytes, chondrocytes and neurons (5,6), which regulate the immune response and are important in tissue repair, thus attracting attention for potential as a novel therapeutic approach (7,8). Compared with other sources of MSCs, adipose tissue-derived stem cells (ADSCs) have the advantages of being abundant, readily obtainable from liposuction or excised fat and accumulated rapidly for autologous transplantation with minimal immunogenicity and improved immunoregulatory ability (9). These characteristics make ADSCs more attractive for clinical usage in cell-based therapy. Previous studies have demonstrated the immunoregulatory roles of ADSCs in tissue and organ repair (10-12), particularly on the protective effects of ADSCs on acute or chronic liver failure and liver fibrosis (13,14). However, the liver injury model used in these investigations was induced by chemical drug treatments, including tetrachloromethane, d-galactosamine and thioacetamide. Therefore, the underlying mechanisms and the pathological changes observed were different to those observed in the most common liver diseases, including viral-induced (hepatitis B or C) chronic inflamma-

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tion and fatty liver disease (FLD) (15,16). As one of the most common forms of FLD, NAFLD refers to a series of pathological changes, including hepatic steatosis, NASH, liver fibrosis and cirrhosis, which are not easily mimicked by chemical drug treatment. Administration of a high-fat-diet (HFD) is usually used to establish NAFLD animal models (17), which more closely resembles the clinical pathological progression of NAFLD disease.

In the present study, in order to investigate whether transplantation with ADSCs attenuates disease progression at an early stage of NAFLD, intrahepatic transplantation of ADSCs was performed on an HFD-induced NAFLD rat model, and the liver function and histopathological characteristics of the liver following transplantation were carefully evaluated.

Materials and methods

Animal experiments. A total of 40 adult male Sprague-Dawley rats (weight, 180–200 g) were obtained from the Center for Animal Experiment of Fujian Medical University (Fuzhou, China; license no. SCXKmin 2012-0002) and were housed at constant temperature ($22\pm 2^{\circ}\text{C}$) and 60% relative humidity, with a 12:12 h light-dark cycle. Rats had *ad libitum* access to food and autoclaved water. All animal procedures were approved by the Animal Ethics Committee of Fuzhou General Hospital of the Fujian province of China (Fuzhou, China).

Isolation and culture of rat ADSCs. Male Sprague-Dawley rats were anesthetized with 2% pentobarbital sodium (40 mg/kg; wt; i.p.; Merck Chemicals, Shanghai, China), and the adipose tissues ($\sim 3\times 1.5\times 0.5$ cm) were collected by excision from the subcutaneous inguinal region of male Sprague-Dawley rats, and washed thoroughly with phosphate-buffered saline (PBS; GE Healthcare Life Sciences, Logan, UT, USA). Subsequently, the tissues were cut into small sections ($\sim 0.1\text{mm}^3$) and digested with 0.1% type I collagenase (Sigma-Aldrich, St. Louis, MO, USA) at 37°C with gentle agitation for 60 min. Subsequently, the type I collagenase activity was neutralized by adding an equal volume (10 ml per rat) of α -modified Eagle's minimum essential medium (α -MEM; GE Healthcare Life Sciences) containing 20% fetal bovine serum (FBS; Gibco Life Technologies, Carlsbad, CA, USA), followed by filtering through an $100\text{ }\mu\text{m}$ cell strainer and centrifugation at $400\times g$ for 10 min at room temperature. The supernatant containing the lipid droplets was discarded and the cell pellet was washed twice with PBS. The cell pellets containing the erythrocytes were then lysed with osmotic lysates (Biyuntian Biotech Co., Ltd., Shanghai, China). The remaining cells were suspended in the complete medium, consisting of α -MEM, with 20% FBS, supplemented with 1% penicillin/streptomycin (Gibco Life Technologies). Subsequently, the resuspended cells were immediately transferred into 25 cm^2 cell culture flasks (Corning Incorporated, Corning, NY, USA) at a density of 1×10^6 cells/ml, and incubated at 37°C with 5% CO_2 . The medium was replaced after 24 h and the adherent cells were further expanded with complete medium, which was replaced every 2 days. Once the cells had reached $\sim 80\%$ confluence, they were detached using 0.25% trypsin-0.02% EDTA (Gibco Life Technologies) and passaged at a dilution of 1:3. Cultured cells at the third

passage were used for the analysis of cell surface markers and for transplantation into the livers of the NAFLD rats.

Flow cytometry analysis. The surface biomarkers of the ADSCs were characterized using flow cytometry to ensure cell quality. The cultured cells were detached using 0.25% trypsin/0.02% EDTA and were suspended in PBS containing 5% bovine serum albumin (Sigma-Aldrich). Subsequently, the cells were incubated with the following primary antibodies for 60 min at room temperature: PE-conjugated anti-mouse/rat CD29 (monoclonal; 1:200; eBioscience, San Diego, CA, USA; cat. no. 12-0291-82), mouse anti-rat/human CD31 (monoclonal; 1:100; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; cat. no. SC-80913), mouse anti-rat/human CD34 (monoclonal; 1:100; Santa Cruz Biotechnology, Inc.; cat. no. SC-7324), PE-conjugated anti-mouse/rat CD44H (monoclonal; 1:200; eBioscience; cat. no. 12-0444-82), rabbit anti-rat/human/mouse CD45 (polyclonal; 1:100; Santa Cruz Biotechnology, Inc.; cat. no. SC-25590) and rabbit anti-rat/human/mouse CD90 (polyclonal; 1:100; Santa Cruz Biotechnology, Inc.; cat. no. SC-9163). The cells were then washed twice with PBS and further incubated with fluorescent-conjugated secondary antibodies (donkey anti-mouse IgG-Alexa Fluor[®] 488 (polyclonal, 1:1,000) or donkey anti-rabbit IgG-Alexa Fluor[®] 647 (polyclonal, 1:1,000), obtained from Invitrogen Life Technologies (Carlsbad, CA, USA; cat. no. A-21202 or A31573) for 30 min at room temperature. Finally, the cells were washed twice with PBS and characterized using a fluorescence-activated cell sorter (FACS; BD Biosciences, Franklin Lakes, NJ, USA). The raw data was further analyzed using FlowJo 7.6 software (FlowJo, LLC, Ashland, OR, USA).

Transplantation of ADSCs into the livers of NAFLD rats. The NAFLD rat model was induced using a previously described procedure (17) with modification. Briefly, the rats were fed either with normal chow (control group) or with a HFD, which contained 88% normal chow, 2% cholesterol and 10% lard, for 6 weeks. Subsequent to the development of hepatic steatosis, the NAFLD rats ($n=20$) were randomly divided into two groups: The NAFLD group ($n=10$), treated with PBS (1 ml/rat), as a mock-treatment group; and the ADSCs therapy group ($n=10$), which received intrahepatic transplantation of ADSCs (2×10^6 cells/rat). The transplantation was performed as follows: The rats were temporarily anesthetized with diethyl ether (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), following which the abdominal cavity was opened under aseptic conditions, and the portal vein was exposed using moistened swabs. Subsequently, the suspension of ADSCs in PBS was injected into the portal vein using a 24-gauge needle. All NAFLD rats were continuously fed with HFD and were sacrificed with 2% pentobarbital sodium (100 mg/kg; wt; i.p.) (Merck Chemicals) 2 or 4 weeks after transplantation. The liver tissues ($\sim 400\text{mg}$ per rat) and sera (~ 3 ml per rat) were simultaneously collected for further investigation.

Biochemical assays of liver function. The collected sera was separated by centrifugation at $1,000\times g$ at 4°C for 10 min, then was stored at -80°C for further use. The serum levels of total cholesterol (TC), triglycerides (TG), fatty acids (FA), alanine

aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were measured using TC, TG, FA, ALT, AST and TBIL ELISA kits, respectively, according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

To further investigate whether ADSC transplantation improved hepatic lipid peroxidation, each liver sample was homogenized in stroke-physiological saline solution (Fuzhou Neptunus Futao Pharmaceuticals Co., Ltd, Fuzhou, China), and was then centrifuged at 5,000 x g for 10 min at 4°C. The supernatants were collected and the activity of liver superoxide dismutase (SOD), and the content of malondialdehyde (MDA) were measured using SOD and MDA assay kits (Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's instructions. The activity of SOD and the MDA content were normalized to the level of total protein, which was measured using a bicinchoninic acid assay kit (Beijing TransGen Biotech Co., Ltd., Beijing, China).

Histopathological assessment. The obtained liver tissues were fixed in 4% paraformaldehyde for 24 h, then were gradually dehydrated with ethanol, embedded in paraffin and stained with hematoxylin and eosin (H&E) staining kit (Jiancheng Bioengineering Institute, Nanjing, China). The presence of hepatic steatosis and inflammation evaluated by two pathologists in a double-blinded manner. To further visualize the accumulation of fat in the liver, oil red O staining was performed using a commercial kit (Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's instructions. The histopathological examination was performed using an Olympus microscope (BX51; Tokyo, Japan).

Assessment of pro-inflammatory cytokines. The secretion levels of tumor necrosis factor (TNF)- α , interleukin (IL)-6, cyclooxygenase (COX)-2, IL-1 β , monocyte chemoattractant protein (MCP)-1 and interferon (IFN)- γ in the serum were evaluated using ab ELISA (Shanghai BlueGene Biotech Co., Ltd., Shanghai, China), according to the manufacturer's instructions. Briefly, the standards and samples were added to an antibody-coated 96-well plate, incubated at 37°C for 1 h and, followed with double-distilled water 3 times, the substrate was then added to each well at 37°C for 10 min. Absorbance values were measured at 450 nm using a SpectraMax M5e microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Statistical analyses. GraphPad Prism, version 6.00 (GraphPad Software, Inc., La Jolla, CA, USA) for Windows was used for statistics analysis. All quantitative data are expressed as the mean \pm standard deviation. Statistical analysis among different groups was performed using Student's t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characterization of rat ADSCs. The derived ADSCs were first characterized by their fibroblast-like morphology, which is consistent with the morphological characteristics of ADSCs from other species (Fig. 1A) (13). Subsequently, the specific

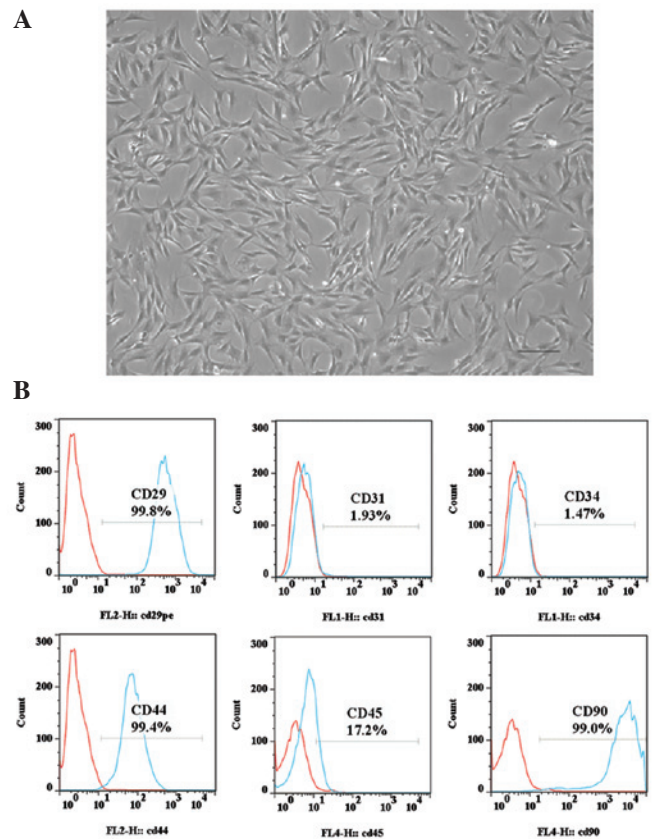


Figure 1. Characterization of rat ADSCs. (A) Morphology of rat ADSCs at passage three (magnification, x100). (B) Flow cytometry demonstrated that CD29, CD44 and CD90 were positively expressed, CD45 was moderately expressed and CD31 and CD34 were negatively expressed in the rat ADSCs. Red lines indicate the negative control, while blue lines indicate the expression of surface biomarkers. ADSCs, adipose tissue-derived stem cells.

surface biomarkers of ADSCs were characterized using fluorescence-activated cell sorting, in which CD29 (99.8%), CD44 (99.4%) and CD90 (99.0%) were positively expressed, CD45 (17.2%) was moderately expressed, and CD31 (1.93%) and CD34 (1.47%) were negatively expressed (Fig. 1B). This expression pattern of the surface biomarkers was consistent with that reported for characterization of ADSCs (14). Therefore, the derived cells were deemed suitable for further transplantation experiments.

ADSC transplantation improves the hepatic functions of NAFLD model rats. To confirm whether transplantation of ADSCs was able to attenuate hepatic injuries, serum levels of ALT, AST and TBIL, which are associated with liver function, were measured 2 or 4 weeks following ADSC therapy. As shown in Fig. 2, the serum levels of ALT and TBIL in the NAFLD rats were significantly higher following 2 weeks of further feeding with an HFD (8 weeks HFD in total), compared with the normal rats, however, the AST level was not significantly different. On further feeding of NAFLD rats with HFD for 4 weeks (10 weeks HFD in total), the serum levels of ALT, TBIL and AST were significantly higher, compared with the normal rats, indicating severe damage to the liver function of NAFLD rats. At 2 weeks post-ADSC transplantation into the NAFLD rats, the serum levels of ALT, TBIL and AST were all significantly reduced, almost

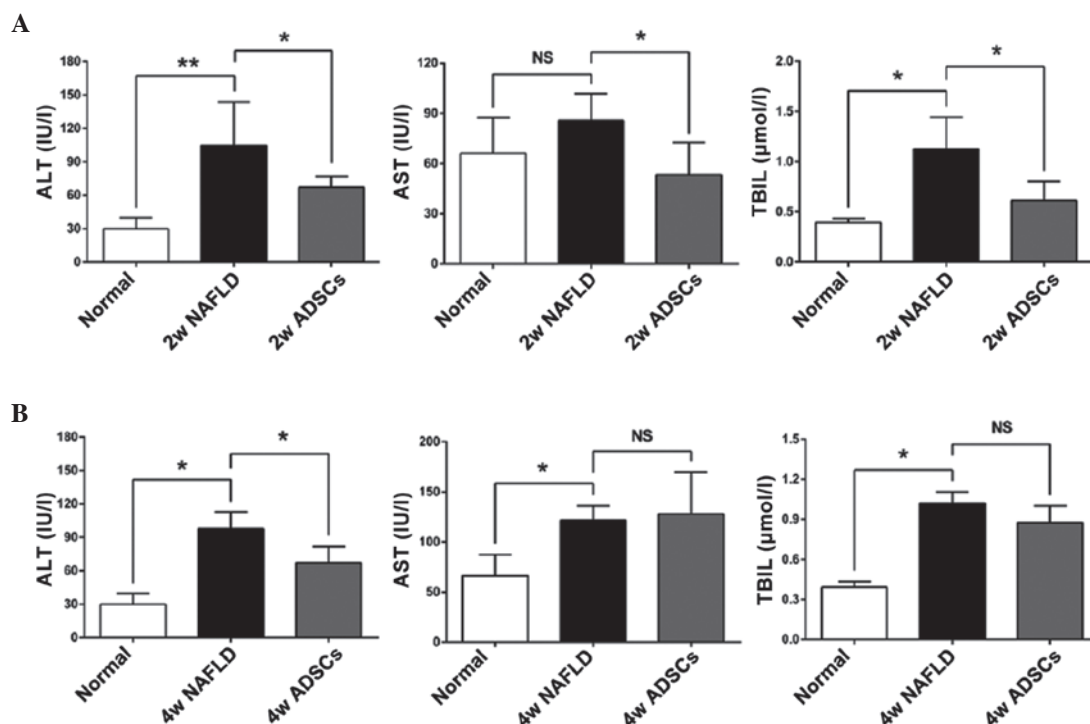


Figure 2. Serum levels of ALT, AST and TBIL following ADSC transplantation. At (A) 2 or (B) 4 weeks post-ADSC transplantation, the serum levels of ALT, AST and TBIL were measured by ELISA. Compared with the NAFLD group, the ADSC groups exhibited significantly reduced serum levels of ALT, AST and TBIL 2 weeks after transplantation, however, only ALT was reduced 4 weeks after transplantation. For all groups, $n=5$. * $P<0.05$; ** $P<0.01$. Normal, untreated rats; 2w/4w NAFLD, NAFLD rats fed a high fat diet (HFD) for a further 2/4 weeks; 2w/4w ADSCs, 2/4 weeks after intrahepatic transplantation of ADSCs into NAFLD rats. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ADSCs, adipose tissue-derived stem cells; NAFLD, non-alcoholic fatty liver disease; HFD, high-fat-diet; NS, not significant.

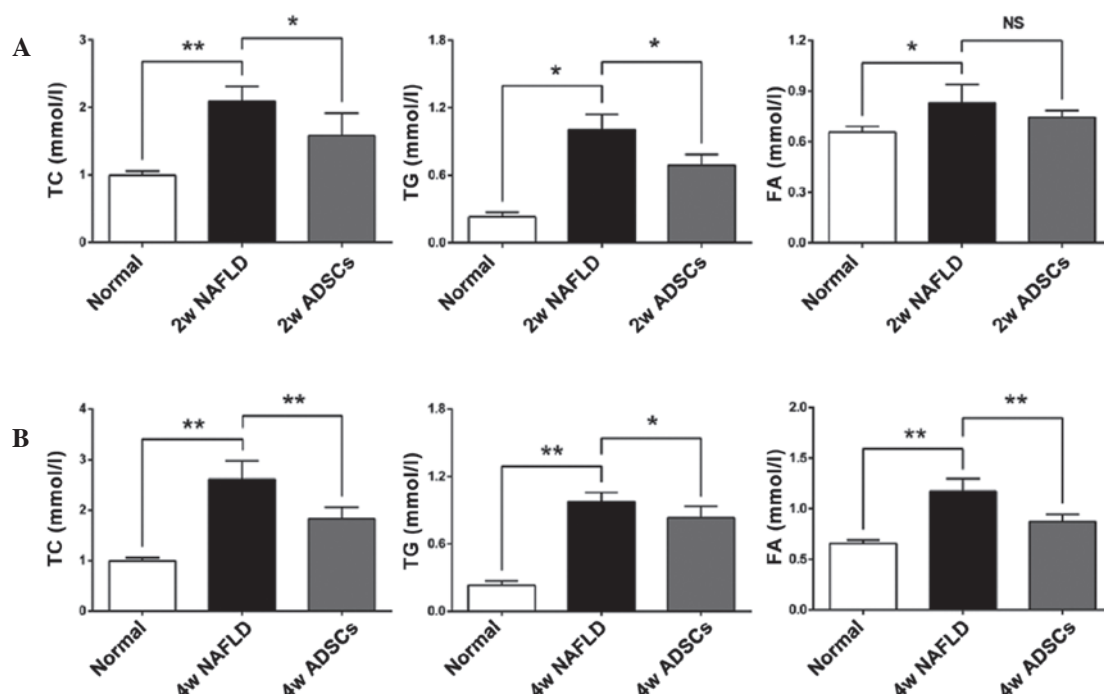


Figure 3. Serum levels of FA, TG and TC following transplantation of ADSCs. The serum levels of TC, TG and FA were measured (A) 2 weeks or (B) 4 weeks after ADSCs transplantation. Compared with the NAFLD groups, transplantation with ADSCs significantly reduced the serum levels of TC and TG at 2 and 4 weeks post-transplantation. The serum level of FA was only reduced 4 weeks after transplantation. For all groups, $n=5$. Data are expressed as the mean \pm standard deviation. * $P<0.05$; ** $P<0.01$. FA, fatty acids; TG, triglycerides; TC, total cholesterol; ADSCs, adipose tissue-derived stem cells; NAFLD, non-alcoholic fatty liver disease; NS, not significant; Normal, untreated rats; 2w/4w NAFLD, NAFLD rats fed high fat diet for a further 2/4 weeks; 2w/4w ADSCs, 2/4 weeks after intrahepatic transplantation of ADSCs into NAFLD rats.

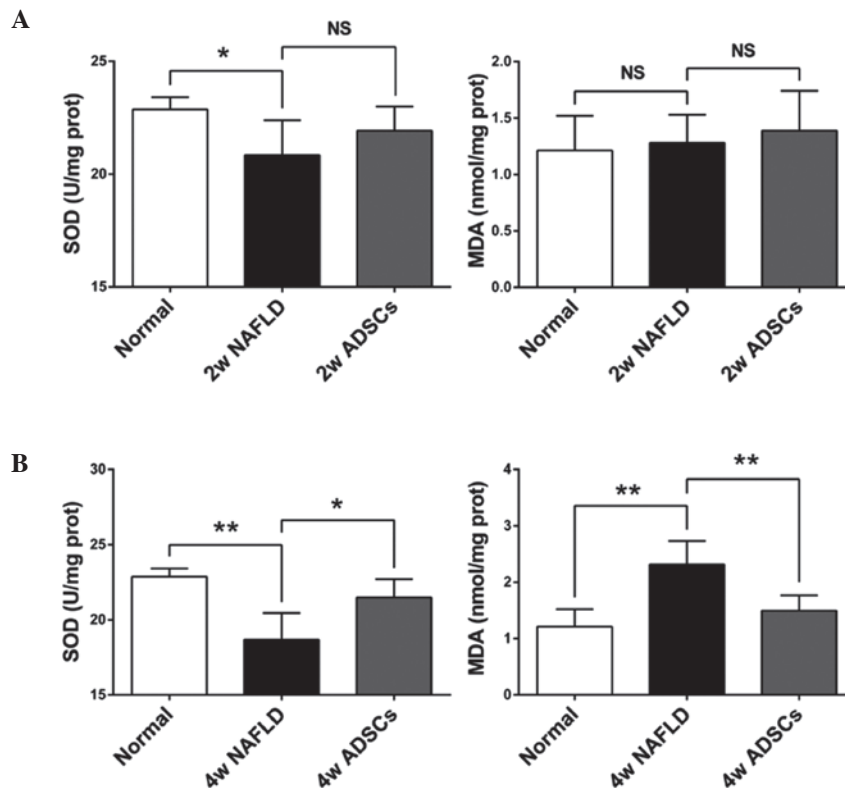


Figure 4. SOD activity and MDA content in liver tissues following transplantation of ADSCs. The liver homogenate levels of MDA and SOD activity, which are closely associated with oxidative stress, were measured following (A) 2 or (B) 4 weeks of ADSCs transplantation. Compared with the NAFLD groups, ADSCs were able to significantly reduce the MDA contents and increase the SOD activity following 4 weeks of transplantation. For all groups, $n=5$. Data are expressed as the mean \pm standard deviation. * $P<0.05$; ** $P<0.01$. SOD, superoxide dismutase; MDA, malondialdehyde; ADSCs, adipose tissue-derived stem cells; NAFLD, non-alcoholic fatty liver disease; NS, not significant; Normal, untreated rats; 2w/4w NAFLD, NAFLD rats fed a high fat diet for a further 2/4 weeks; 2w/4w ADSCs, 2/4 weeks after intrahepatic transplantation of ADSCs into NAFLD rats.

to the levels observed in the normal rats, which demonstrated that transplantation with ADSCs protected the liver function against damage. However, only ALT remained significantly lower 4 weeks after ADSC transplantation, compared with the NAFLD rats. The levels of TBIL and AST were no longer significantly different compared with the NAFLD rats, possibly due to the continuous HFD feeding during the treatment of all rats.

Transplantation with ADSCs promotes lipid metabolism in NAFLD rats. Disorder of lipid metabolism is the predominant biochemical change in the development of NAFLD (1). To determine whether ADSC transplantation promoted the lipid metabolism in the livers of NAFLD rats, the serum levels of TC, TG and FA were measured 2 or 4 weeks after transplantation with the ADSCs. As shown in Fig. 3, higher levels of TC, TG and FA were observed in the NAFLD rats, compared with the normal rats, which indicated that the NAFLD rats had imbalanced lipid metabolism. At 2 weeks post-ADSC transplantation in the NAFLD rats, the serum levels of TC and TG were significantly reduced, while the serum level of FA was not significantly reduced, compared with the NAFLD rats. However, at 4 weeks post-ADSC transplantation, the serum levels of TC, TG and FA were all significantly lower than those in the NAFLD rats, suggesting that the transplanted ADSCs promoted lipid metabolism.

Transplantation with ADSCs reduces oxidative stress in the liver tissues. To further examine the effect of ADSC transplantation on oxidative stress in the livers of NAFLD rats, the SOD activity and MDA content in the liver homogenates were evaluated. As shown in Fig. 4, the activity of SOD and MDA content in the NAFLD rats, which received another 2 weeks HFD feeding (8 weeks HFD in total) were not significantly different, compared with the normal rats. However, the SOD activity was significantly reduced and the MDA content was significantly increased in the NAFLD rats, which received another 4 weeks HFD feeding (10 weeks HFD in total), compared with the normal rats, indicating that oxidative stress was induced in the NAFLD rats. At 2 weeks post-ADSC transplantation in the NAFLD rats, the SOD activity and MDA content were not significantly altered. However, the SOD activity was significantly higher and the MDA content was significantly lower, compared with the NAFLD rats, at 4 weeks post-ADSC transplantation. These results suggested that ADSC transplantation reduces oxidative stress in the liver.

ADSCs transplantation decelerates hepatic pathological changes in NAFLD rats. Hepatic histopathological assessment is the gold standard for NAFLD diagnosis (18). In order to determine whether transplantation with ADSCs has a protective role by slowing pathological progression, the liver tissues were evaluated following H&E staining. As shown in Fig. 5,

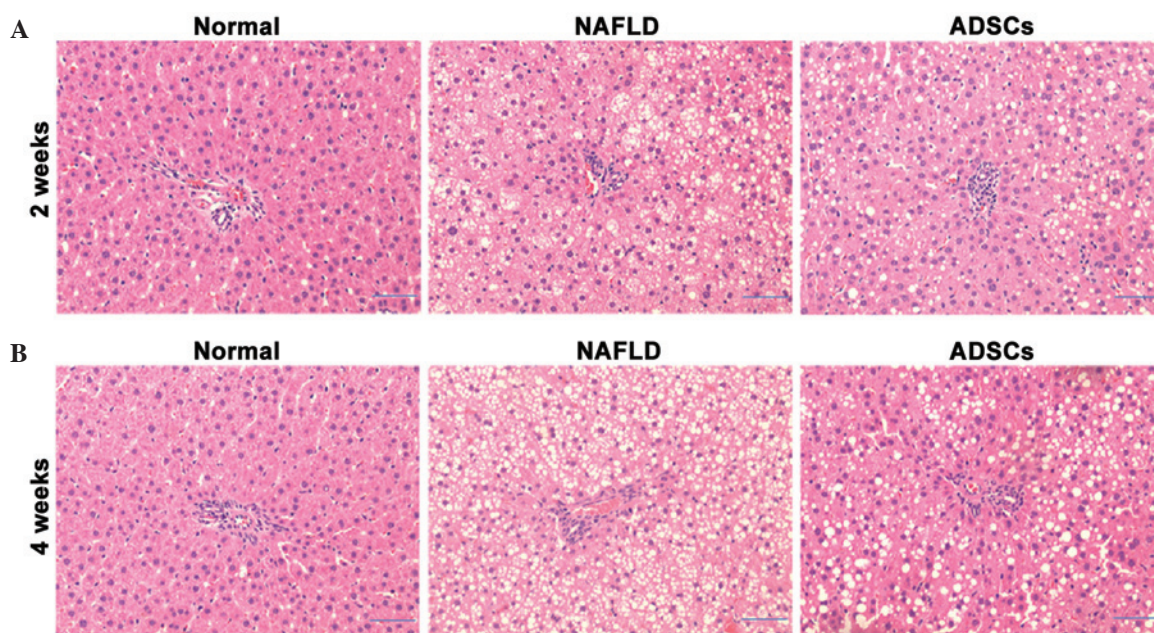


Figure 5. Hepatic pathological changes following ADSC transplantation. At (A) 2 or (B) 4 weeks post-ADSC transplantation, the liver tissues from normal rats, NAFLD rats and ADSC-treated rats were evaluated using hematoxylin and eosin staining (magnification, x100). The normal rats exhibited no evidence of steatosis or inflammation. (A) NAFLD rats appeared to exhibit typical steatosis, whereas few incidences of steatosis were observed 2 weeks post-ADSC transplantation. (B) Severe steatosis was observed in the NAFLD rats, while low grade steatosis was observed 4 weeks post-ADSCs transplantation. ADSCs, adipose tissue-derived stem cells; NAFLD, non-alcoholic fatty liver disease.

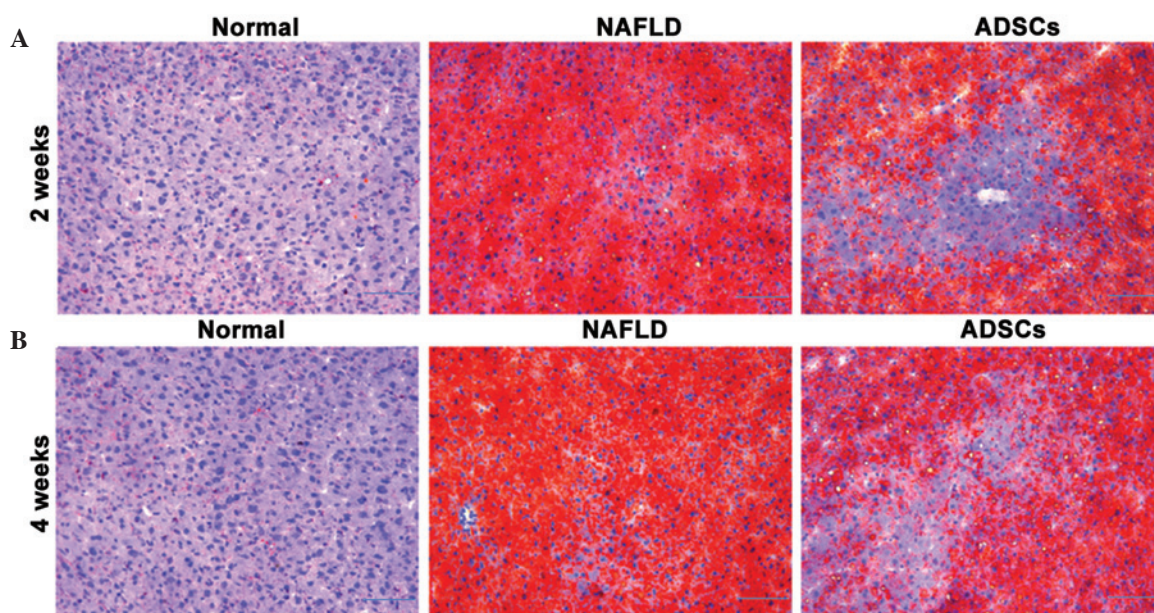


Figure 6. Lipid accumulation in the liver following ADSC transplantation. At (A) 2 or (B) 4 weeks post-ADSC transplantation, levels of lipid accumulation in the normal, NAFLD and ADSC-treated rats were evaluated using oil red O staining (magnification, x200). No evidence of lipid accumulation was observed in the normal rats. Increased lipid accumulation was observed in the NAFLD rats, while lipid accumulation was significantly reduced following ADSC transplantation. ADSCs, adipose tissue-derived stem cells; NAFLD, non-alcoholic fatty liver disease.

no evidence of steatosis or inflammation was identified in the liver tissues of the normal group. Following HFD feeding, the NAFLD group exhibited typical steatosis, indicating that the NAFLD model was successfully established. However, evidence of steatosis was limited 2 weeks post-ADSC transplantation, and low grade-steatosis was observed 4 weeks post-ADSC transplantation, which indicated that the ADSCs attenuated the development of NAFLD.

The excessive accumulation of hepatic lipids is the major pathological feature in the progression of NAFLD (19). To further confirm whether transplantation with ADSCs reduced lipid accumulation in the liver tissues, the hepatic lipid contents were evaluated using oil red O staining. As shown in Fig. 6, no lipid accumulation was observed in the normal rats, however, numerous lipids were observed in the liver tissues of the NAFLD rats. The ADSC group exhibited lower lipid

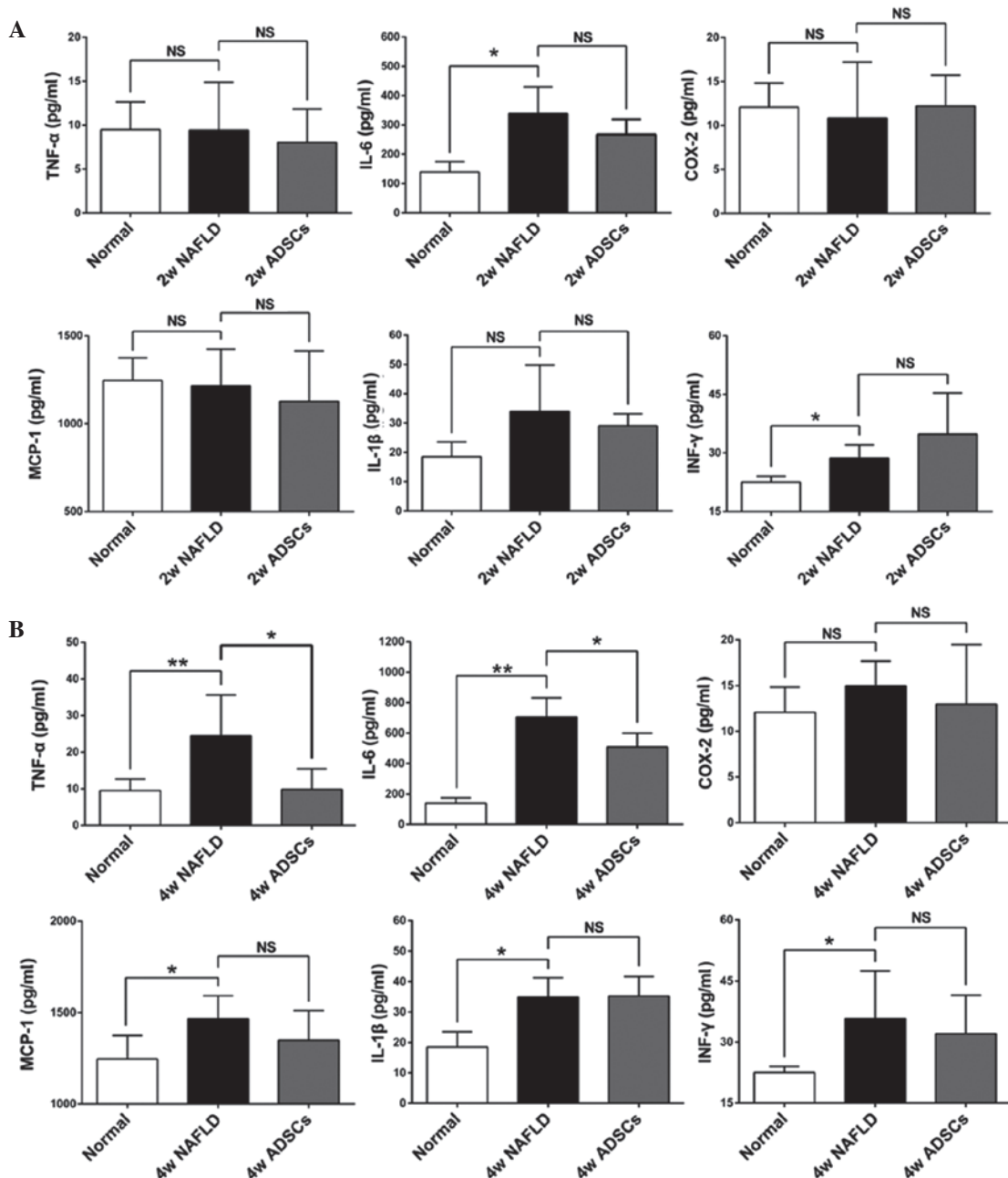


Figure 7. Serum levels of pro-inflammatory cytokines following ADSC transplantation. The serum levels of TNF- α , IL-6, COX-2, IL-1 β , MCP-1 and INF- γ were measured by ELISA (A) 2 or (B) 4 weeks after ADSC transplantation. Compared with the NAFLD rats, significantly reduced the serum levels of TNF- α and IL-6 were observed 4 weeks after transplantation. For all groups, n=5. Data are expressed as the mean \pm standard deviation. *P<0.05; **P<0.01. ADSCs, adipose tissue-derived stem cells; TNF- α , tumor necrosis factor- α ; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; INF- γ , interferon- γ ; NAFLD, non-alcoholic fatty liver disease; NS, not significant; Normal, untreated rats; 2w/4w NAFLD, NAFLD rats fed a high fat diet for a further 2/4 weeks; 2w/4w ADSCs, 2/4 weeks after intrahepatic transplantation of ADSCs into NAFLD rats.

accumulation, compared with the NAFLD rats, suggesting that the ADSCs reduced lipid accumulation in the livers of NAFLD rats, thus decelerating disease progression.

ADSC transplantation selectively reduces the serum levels of TNF- α and IL-6 in NAFLD rats. Inflammation is important in the pathogenesis of hepatic steatosis to NASH. To further assess whether transplantation with ADSCs had an immunoregulatory role by reducing the secretion of pro-inflammatory cytokines, the serum levels of TNF- α , IL-6, COX-2, IL-1 β , MCP-1 and INF- γ were detected by ELISA. As shown in Fig. 7, the serum levels of IL-6 and INF- γ were significantly

increased in the NAFLD rats, which received another 2 weeks HFD feeding (8 weeks HFD in total), while no significant differences were observed in the other cytokines between this group and the normal rats. This indicated that the rat livers were not undergoing inflammation. However, following 4 weeks HFD feeding of the NAFLD rats (10 weeks HFD in total), the serum levels of TNF- α , IL-6, IL-1 β , MCP-1 and INF- γ were all significantly increased, with no significant change in COX-2 levels, compared with the normal rats. These data suggested that the rat livers were undergoing inflammation. At 2 weeks post-ADSC transplantation, none of the measured cytokines were significantly different,

compared with the NAFLD rats, due to the fact that inflammation was not present in the rat livers. Notably, the serum levels of IL-6 and TNF- α , which are predominantly secreted by the kuffer cells in the liver and actively contribute to the acute inflammatory responses (20,21), were significantly reduced at 4 weeks post-ADSC transplantation, compared with the NAFLD rats, although no significant changes were observed in the other cytokines measured. Taken together, these results demonstrated that ADSC transplantation selectively reduced the secretion of pro-inflammatory cytokines, including TNF- α and IL-6, attenuating the progression of NAFLD.

Discussion

MSC transplantation is being investigated as an alternative therapeutic approach for liver transplantation in chronic liver diseases (22). Previous studies have demonstrated that multi-potent stromal cells and the hepatocyte-like cells from bone marrow-derived stem cells may offer a potential solution to prevent the progression of NAFLD in animal models (23,24). However, whether ADSCs have a therapeutic effect on NAFLD remains to be fully elucidated. In the present study, ADSCs were able to efficiently protect the NAFLD rats from disease progression, which suggested that ADSC transplantation may be an effective therapeutic approach for NAFLD.

According to the 'two-hit' hypothesis, the 'first hit' is marked by the accumulation of hepatic lipids, but not due to the alcohol abuse (1,4,25), following which liver function is damaged. In the present study, transplantation with ADSCs improved liver function through reductions in ALT activity and total TBIL (Fig. 2), in addition to promoting lipid metabolism (Fig. 3). These observations were consistent with the hepatic pathological morphological changes in the H&E (Fig. 5) and oil red O staining (Fig. 6), confirming that ADSCs attenuated the disease progression of NAFLD.

Under pathological conditions, excessive lipid accumulations can cause damage via peroxidation, resulting in the occurrence of marked oxidative stress (26-28). In the present study, transplantation with ADSCs reduced oxidative stress by increasing the activity of SOD and reducing the content of MDA (Fig. 4), which may result from the suppression of lipid peroxidation by ADSC transplantation.

It has been demonstrated that inflammation is important in the progression of NAFLD (26,29,30). In the present study, high levels of pro-inflammatory cytokines, including IL-6, TNF- α , IL-1 β , MCP-1 and IFN- γ were observed in the NAFLD rats, which suggested that inflammation was induced in the model. However, the secretions of IL-6 and TNF- α , which are considered to be major inflammatory mediators of steatosis, insulin resistance and associated inflammatory disorders (31,32), were significantly inhibited following ADSC transplantation. These results suggested that ADSC transplantation selectively inhibited inflammation, mediated by IL-6 and TNF- α . Notably, the level of COX-2 was not increased in the NAFLD rats, compared with the normal rats, which may be due to the possibility that the animal model had not progressed to NASH, as significant increases in COX-2 are reported to appear only in the NASH stage (33,34).

Certain key parameters, including serum TBIL and AST were only reduced 2 weeks after transplantation with ADSCs, however, these changes were not observed 4 weeks after transplantation. This may be due to continued HFD feeding attenuating the therapeutic effects of ADSC transplantation. To more effectively prevent NAFLD progression, intrahepatic transplantation may be combined with multiple supplementary injections of ADSCs via the tail or penis vein, to further maintain the therapeutic effects.

ADSCs may be more suitable for clinical use due to the advantages of being abundant, readily maintained, highly proliferative and exhibiting improved immunoregulatory activities (9,35), however, further investigation into the possible tumorigenic risk are to be performed prior to clinical application. In the present study, the application of ADSC transplantation reduced the rate of disease progression in the NAFLD rat model. The results demonstrated that ADSC transplantation significantly improved liver function, reduced lipid accumulation and attenuated the inflammatory state of the liver, therefore decelerating the progression of NAFLD in the rat model. In conclusion, ADSC transplantation may offer a promising therapeutic approach for the treatment of NAFLD in the future.

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