Immediate Intraportal Transplantation of Human Bone Marrow Mesenchymal Stem Cells Prevents Death From Fulminant Hepatic Failure in Pigs

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The effectiveness of human bone marrow mesenchymal stem cell (hBMSC) transplantation to treat acute and chronic liver injury has been demonstrated in animal models and in a few nonrandomized clinical trials. However, no studies have investigated hBMSC transplantation in the treatment of fulminant hepatic failure (FHF), especially in large animal (pig) models. The aim of this study was to demonstrate the safety, effectiveness, and underlying mechanism of hBMSC transplantation for treating FHF in pigs through the intraportal route. Human BMSCs ($3 \times 10^7$) were transplanted into pigs with FHF via the intraportal route or peripheral vein immediately after D-galactosamine injection, and a sham group underwent intraportal transplantation (IPT) without cells (IPT, peripheral vein transplantation [PVT], and control groups, respectively, n = 15 per group). All of the animals in the PVT and control groups died of FHF within 96 hours. In contrast, 13 of 15 animals in the IPT group achieved long-term survival (>6 months). Immunohistochemistry demonstrated that transplanted hBMSC-derived hepatocytes in surviving animals were widely distributed in the hepatic lobules and the liver parenchyma from weeks 2 to 10. Thirty percent of the hepatocytes were hBMSC-derived. However, the number of transplanted cells decreased significantly at week 15. Only a few single cells were scattered in the regenerated liver lobules at week 20, and the liver tissues exhibited a nearly normal structure. Conclusion: Immediate IPT of hBMSCs is a safe and effective treatment for FHF. The transplanted hMSCs may quickly participate in liver regeneration via proliferation and transdifferentiation into hepatocytes during the initial stage of FHF. This method can possibly be used in future clinical therapy.

End-stage hepatic failure is a potentially life-threatening condition for which orthotopic liver transplantation is the only effective treatment. However, a shortage of available donor organs for transplantation results in the death of many patients awaiting liver transplantation. Hepatocyte transplantation provides a promising alternative, and numerous experiments have demonstrated that hepatocyte transplantation improves liver function in animals with hepatic failure and innate liver-based metabolic disorders. However, hepatocyte transplantation has rarely produced therapeutic effects, because mature hepatocytes cannot be effectively expanded in vitro and the availability of hepatocytes is often limited by shortages of donor organs. Thus, previous studies have focused on the development of various stem cells that could be readily isolated using noninvasive procedures to yield hepatocytes in vitro and in vivo. Bone marrow mesenchymal stem cells (BMSCs) can differentiate into osteoblasts, adipocytes, and other mesenchymal cell lineages. The hematopoiesis differentiation capacity of human BMSCs (hBMSCs) has been

**Abbreviations:** ALB, albumin; ALT, alanine aminotransferase; BMSMC, bone marrow mesenchymal stem cell; D-gal, D-galactosamine; ELISA, enzyme-linked immunosorbent assay; FHF, fulminant hepatic failure; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; hBMSC, human BMSC; H&E, hematoxylin and eosin; HNF-1a, hepatocyte nuclear factor-1a; HSA, hepatocyte-specific antigen; IPT, intraportal transplantation; PVT, peripheral transplantation; qPCR, quantitative real-time polymerase chain reaction.

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characterized in vitro and in vivo.11-13 These cells can also be expanded in culture for long periods without any apparent loss of differentiation capacity. Some groups have already started transplanting autologous bone marrow cells into patients with chronic liver fibrosis or cirrhosis.12,14,15 However, little is known about the use of hBMSCs to treat fulminant hepatic failure (FHF) in animal models or in human patients with FHF, even though such studies would be clinically important.5 Furthermore, because of difficulties in tracking transplanted hBMSC-derived hepatocytes in patients, and because previous experiments were performed in small animal (mouse or rat) models of chronic liver injury, the roles of BMSCs in liver regeneration have not been fully elucidated.5 FHF-derived BMSCs demonstrate a hepatic transcriptional profile and express many of the same genes expressed by hepatic progenitor cells,16-18 suggesting that extrahepatic stem cells, especially BMSCs, may be a resource for hepatocyte repopulation and can play an important role in liver regeneration. Thus, we investigated whether the intraportal transplantation of hBMSCs is a safe and effective method to prevent FHF in a large animal (pig) model.

Materials and Methods

Isolation and Culture of hBMSCs. Human BMSCs were isolated by bone marrow aspiration from the iliac crest of 30 healthy male volunteers. Signed informed consent was obtained, and the protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University. Bone marrow mononuclear cells were purified by Ficoll-Paque density-gradient centrifugation as described.16 The purified mononuclear cells were allowed to attach in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) overnight at 37°C in 5% CO2. Floating cells were washed out on the second day, and all attached cells were maintained using the same culture medium. The cells from passages 3-5 were used for subsequent experiments.

Phenotypic Analysis via Flow Cytometry. Pheno-
typic analyses of cultured hBMSCs were performed prior to transplantation via standard flow cytometry methods. The third and fifth passages of the hBMSCs (1 × 10⁶ cells) were incubated with direct phycoerythrin- or fluorescein isothiocyanate–conjugated mouse monoclonal antibodies against human CD34 (Santa Cruz Biotechnology, Santa Cruz, CA), CD45, CD29 (both from Abcam, Cambridge, UK), and CD90 (BD Biosciences, San Jose, CA) for 60 minutes in the dark at 4°C, followed by washing and resuspension in phosphate-buffered saline. Immunoglobulin isotype incubation was performed as a negative control. Flow cytometry was performed with a FACSCalibur system (FC500, Beckman Coulter, Fullerton, CA).

Multilineage Differentiation Assays. To induce osteogenic differentiation, hBMSCs were cultured in a commercially available osteogenic differentiation medium (Cambrex, Walkersville, MD). On day 21, the alkaline phosphatase activity of the cultured cells was assessed as described.19 To induce adipogenic differentiation, hBMSCs were cultured in a commercially available adipogenic differentiation medium purchased from Cambrex. On day 21, cells were stained with Oil red O. Hepatogenic differentiation was performed as described.17 On day 21, the cultured cells were characterized via quantitative real-time polymerase chain reaction (qPCR) with hepatic-specific gene primers [albumin (ALB), cytokeratin 8 (CK8), glucose-6-phosphate dehydrogenase (G6PD) and hepatocyte nuclear factor-1α (HNF-1α)], whose sequences are provided in Supporting Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control.

FHF Induction and hBMSC Transplantation. All experimental protocols were approved by the Animal Care Ethics Committee of the First Affiliated Hospital, Zhejiang University, and all animals received humane care according to the Guide for the Care and Use of Laboratory Animals. Forty-five male Chinese experimental miniature pigs (Taihe Biotechnology, JiangSu, China) weighing 8-10 kg underwent FHF induction with D-galactosamine (D-gal, Hanhong Chemical, Shanghai, China) at a dose of 1.5 g/kg via jugular vein catheterization as described20 before the cell transplantation procedure. Three groups (n = 15/group) of FHF animals were studied: an hBMSC intraportal transplantation (IPT) group, which received a
transfusion of $3 \times 10^7$ hBMSCs suspended in 10 mL of normal saline via the intrahepatic portal vein under B-ultrasound guidance; an hBMSC peripheral vein transplantation (PVT) group, which received a transfusion of an equal number of cells via the ear vein; and a control group, which received a sham intraportal transplantation procedure with an equal volume of normal saline without cells. No animal received any medical support (e.g., infusions, drugs) during the entire experimental period. The transplantation procedures were all performed by the same B-ultrasound expert with 5 years of extensive experience.

**Long-term Survival and Biochemical Evaluation.** Animals were evaluated for up to 6 months after transplantation. As biochemical markers of liver metabolism, coagulation and hepatocyte damage, alanine aminotransferase (ALT), prothrombin time, total bilirubin, ammonia, blood urea nitrogen, and creatinine levels were analyzed prior to hBMSC transplantation (baseline data) and then on days 1, 2, and 3 and weeks 1, 2, 3, 5, and 8 after transplantation. Survival was analyzed using a Kaplan-Meier plot and log-rank analysis. The data are expressed as the mean ± SD and were evaluated via Student t test and one-way analysis of variance with SPSS software version 16.0 (SPSS, Chicago, IL). The significance for all statistical analyses was defined as $P < 0.05$.

**Histochemistry and Immunohistochemistry.** To determine the effect of the transplanted hBMSCs on liver regeneration, hBMSC-derived hepatocytes engrafted in liver tissues were tracked using immunohistochemistry with the human hepatocyte-specific marker ALB (Bethyl, Montgomery, TX) and a hepatocyte-specific antigen antibody (HSA) (Abcam, Cambridge, UK). Liver tissues were harvested after the pigs died of FHF in the control and PVT groups. In the IPT group, because many unforeseen risks exist in FHF animals that undergo several partial hepatectomies and to ensure an adequate number of surviving animals for follow-up, liver tissue was harvested from five animals. Three liver sections were harvested from each of the left, middle, and right lobes (10-20 g, each sample) via small partial hepatectomy under sterile conditions on weeks 2, 3, 5, 10, 15, and 20 after transplantation. Immunohistochemical analyses of ALB and HSA were performed using serial sections. The hepatectomy procedure was performed by a surgeon with 10 years of experience in liver transplantation. Each liver tissue specimen was analyzed by hematoxylin and eosin (H&E) staining. For H&E staining, each liver tissue section (4-μm-thick) was heat-fixed at 60°C for 1 hour and stained with H&E as described.

For immunohistochemistry, serial tissue sections were applied to poly-l-lysine-coated slides. After the sections were dewaxed, rehydrated, and washed, endogenous peroxidases were inactivated with 3% H$_2$O$_2$ for 10 minutes at room temperature. The sections were incubated overnight with primary anti-human antibodies (ALB, 1:10,000, and HSA 1:1,000) with no cross-reactivity to pig tissues. The sections were washed with phosphate-buffered saline three times and incubated with the appropriate secondary antibodies at 37°C for 1 hour. After reaction with the DAB chromogen, the sections were rinsed with distilled water, counterstained with hematoxylin, and mounted using Histomount (Invitrogen). The labeled cells were visualized with an inverted microscope (Nikon, Eclipse E200, Tokyo, Japan), and digital images were captured using Nis-elements F 3.0 software. Omission of the primary antibody or substitution with an unrelated immunoglobulin G served as negative controls.

**Validating the Hepatogenesis of Transplanted hBMSCs.** To validate the hepatogenesis of transplanted hBMSCs at the level of gene expression, human hepatocyte-specific genes (ALB, CK8, G6PD, and HNF-1z) were analyzed via qPCR (primer sequences are shown in Supporting Table 1) in the same liver tissues used for immunohistochemistry. To evaluate ALB secretion in the surviving animals, the concentration of human ALB (weeks 2, 5, 10, 15, and 20) in the serum of the animals was determined by a competitive enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (BETHYL, Montgomery, TX) and a described protocol.

**Tumorigenicity Assay.** To examine the long-term tumorigenicity of the transplanted hBMSCs, the surviving animals were sacrificed 6 months after cell transplantation, and tissue specimens collected from the brain, heart, lung, kidney, spleen, and pancreas were subjected to histopathological examination.

**Results**

**The Phenotype and Multipotential Stem Cell Characteristics of Transplanted hBMSCs.** The results of the phenotypic analysis by flow cytometry (Supporting Fig. 1) showed that the hBMSCs from passages 3 and 5 were positive for CD29 (98.3% and 95.2%, respectively) and CD90 (98.7% and 96.2%, respectively) but negative for CD34 (1.39% and 1.59%, respectively) and CD45 (1.30% and 1.34%, respectively). These cells exhibited a fibroblast-like morphology (Fig. 1A). The multipotential stem cell characteristics were demonstrated via culture in multilineage differentiation conditions in vitro. The analysis of
alkaline phosphatase activity demonstrated mineralization during osteogenic differentiation in hBMSCs on day 21 (Fig. 1B). The adipogenic differentiation of the hBMSCs was also characterized by Oil red O staining, and lipid droplets were visible in the differentiated adipocytes on day 21 after the induction of differentiation (Fig. 1C). Hepatogenesis was identified by morphology and qPCR. Under phase-contrast microscopy, the differentiated hepatocytes exhibited a polygonal morphology with a low cytoplasm/nucleus ratio (Fig. 1D). The qPCR results show that the differentiated hepatocyte-like cells expressed ALB, CK8, G6PD, and HNF-1α on day 21 after differentiation (Supporting Fig. 2). These results indicate that the cells used for transplantation exhibited the classic hBMSC phenotype and multipotential stem cell characteristics.

**Intraportal Transplantation of hBMSCs Prolongs Survival of FHF Pigs.** During the 6-month follow-up period after cell transplantation, 15 FHF animals in the control group, which received only normal saline via the intraportal vein, survived less than 4 days (2.9 ± 0.2). The transplantation of hBMSCs (3 × 10⁷) via the peripheral vein did not prolong survival beyond 4 days; all 15 animals in the PVT group died within 4 days (3.5 ± 0.1). There was no significant difference between the control and PVT groups (P > 0.05); each animal demonstrated neurological symptoms, such as excitability and cramps, and died. However, after the transplantation of 3 × 10⁷ hBMSCs via the intrahepatic portal vein in the IPT group, 13 of 15 (87.6%) pigs survived for up to 6 months (Fig. 2). Almost all of the long-term survivors demonstrated significantly improved neurological symptoms during the initial 4 days after IPT of hBMSCs. This observation was confirmed by the biochemical and histological examinations. Only two animals survived less than 1 week (5 and 6 days). One animal died from diarrhea on day 5 after transplantation. The subsequent autopsy showed no specific pathological phenomenon in the main organs, including the brain, heart, lung, kidney, spleen and pancreas; however, the liver exhibited FHF pathology. Another animal died on day 6 after transplantation, and the subsequent autopsy revealed that pericardial effusion and the FHF pathology resulted in rapid death.

**Intraportally Transplanted hBMSCs Improve Liver Function in FHF Pigs.** In all three groups, the biochemical evaluation revealed that some biochemical markers of liver function were grossly altered as early as 24 hours after D-gal transfusion in all animals. The ALT, prothrombin time, total bilirubin, and ammonia levels were significantly higher after 24 hours of D-gal induction compared with baseline values (Fig. 3). There were progressive increases of 3.3-fold for ALT, 6.1-fold for prothrombin time, 21.0-fold for total bilirubin, and 3.7-fold for ammonia on day 3 after D-gal and normal saline transfusion, and all of the animals of the control group died within 4 days. Similar progressive increases of these biochemical markers were observed in the PVT group, and all of the animals died within 4 days. However, these biochemical markers showed comparatively lower increases on day 3 (increases of 2.4-fold for ALT, 3.3-fold for prothrombin time, 13.8-fold for total bilirubin, and 2.0-fold for ammonia) in the IPT group, which received
hBMSC transplantation via the intraportal vein. The subsequent follow-up showed that ALT, prothrombin time, total bilirubin, and ammonia decreased to baseline levels at weeks 3, 1, 3, and 2, respectively, after hBMSC transplantation. Thirteen of 15 animals survived for up to 6 months. No significant differences were observed for the biochemical markers blood urea nitrogen and creatinine among the three groups during the initial 3 days or the subsequent 6-month follow-up. Compared with the control and PVT procedures, hBMSC transplantation via the intraportal vein significantly improved liver function and prevented death from FHF.

**Intraportal Transplantation of Human BMSCs Promotes Liver Regeneration.** All D-gal-treated pigs that did not receive intraportal hBMSC transplantation died of FHF after demonstrating jaundice and hemorrhage. FHF was validated by the observation of massive necrosis during histological examination. H&E staining (Fig. 4) showed extensive hepatocyte necrosis with hemorrhaging involving entire lobules. Very few residual hepatocytes were found adjacent to the fibrous septa, and the hepatocytes present had a swollen cytoplasm. There was no evidence of hepatocyte regeneration in any of the specimens. In contrast, new lobules were regenerated in the surviving animals of the IPT group at week 2 after intraportal hBMSC transplantation. Microthrombosis in the sinusoids and other types of microvascular liver necrosis were not observed in the recipient animal livers. After 3 weeks, the surviving animals showed approximately normal liver structures, and no deviations from the normal liver structure were observed at week 5. These data were confirmed by biochemistry.

Transfused hBMSCs were detected by immunohistochemistry with the anti-human hepatocyte-specific antibodies ALB and HSA. The IPT group showed hBMSC-derived hepatocytes in the animal liver tissue. Immunohistochemistry of the serial sections revealed that hBMSC-derived hepatocytes that were positive for...
ALB and HSA were widely distributed in the hepatic lobule at weeks 2, 5, and 10 after IPT (Fig. 5). Approximately 30% of the cells were double-positive cells, and these cells appeared in hepatic lobules as a mass, as small clusters, and as scattered individual cells. This finding indicates that the transplanted hBMSCs were well-integrated in the liver parenchyma. However, the number of transplanted hBMSC-derived hepatocytes was significantly decreased at week 15, and only a few single cells were scattered in the regenerated liver lobules at week 20 (Fig. 6). Thus, the present data demonstrate the capability of adult human BMSCs to differentiate into hepatocytes and repopulate the liver in FHF.

**ALB Secretion and Gene Expression Profile of Transplanted hBMSC-Derived Hepatocytes Validate the Hepatocyte Transdifferentiation of hBMSCs in a Pig FHF Model.** The ELISA results (Fig. 7A) show that the concentration of human ALB in the animals reached 2.02 ± 0.35 g/L at week 2 and increased to 3.88 ± 0.95 g/L and 3.92 ± 0.55 g/L at weeks 5 and 10, respectively. The level of human ALB subsequently decreased to 1.23 ± 0.3 g/L at week 15 and 0.87 ± 0.29 g/L at week 20. The results of qPCR (Fig. 7B) indicate that human hepatocyte-specific genes (ALB, CK8, G6PD, and HNF-1α) were expressed at week 2 after hBMSC IPT. Progressive increases in the expression of these genes were observed within 10 weeks after hBMSC IPT. After 15 weeks, the expression of these genes decreased gradually. Except for a very low level of G6PD transcripts, the expression of these genes was not detectable at week 20.

**Tumorigenicity Assay.** The surviving animals that received hBMSCs via IPT showed no evidence of tumors in the liver during a preliminary tumorigenicity assay at 6 months after transplantation. No tumors were detected in other organs (including the brain, heart, lung, kidney, spleen, and pancreas) by H&E staining.

**Discussion**

Several nonrandomized clinical trials have demonstrated the safety and promising beneficial effects of hBMSCs in the treatment of liver cirrhosis, although the long-term chronic hepatic failure caused by hepatitis B was not markedly improved. However, the value of BMSCs in acute fulminant hepatic failure has not been studied as extensively as that in chronic injuries even though it is also clinically important. In this study, human BMSCs were used to investigate
whether intraportal transplantation is a safe and effective method for generating human hepatocytes and preventing death from FHF. We also investigated whether the route of delivery influences the amount of engrafted hBMSC-derived hepatocytes and their pattern of distribution throughout the parenchyma of the animal liver.

**Safety of the Intraportal hBMSC Transplantation Route.** Within 2-4 days following the induction of FHF in animals, hepatocytes undergo massive necrosis with hemorrhages involving entire lobules, which results in death.20,22 Thus, direct intraportal transfusion within this damaged environment may result in the proliferation and transdifferentiation of transplanted hBMSCs and may stimulate the regeneration of endogenous parenchymal cells. Because the optimal time and route of hBMSC transplantation have not been established and may be as soon as possible after FHF, determining the safety of transplantation is important. In this study, except for two animals that died as a result of severe diarrhea and pericardial effusion on days 5 and 6 after transplantation, the immediate intraportal vein transfusion of hBMSCs successfully prevented the death of 13 animals from FHF, and no animal suffered sudden death. Furthermore, no reactions or rejections were observed in the surviving animals. No tumors developed in the major organs, including the liver, brain, heart, lung, kidney, spleen and pancreas, at six months after the IPT of hBMSCs. A subsequent histologic examination also indicated a lack of microthrombosis in the central vein and peripheral area or microvascular liver necrosis in recipient animal livers during the entire transplantation period. These data suggest that the immediate IPT of hBMSCs is a safe treatment method for FHF.

**Effectiveness of Intraportal hBMSC Transplantation.** The effectiveness of hematopoietic stem cell transplantation in treating acute and chronic liver injury has been demonstrated extensively in animal models23–25 and some initial clinical trials.26–28

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Fig. 6. Immunohistochemistry images at week 15 (serial sections) and week 20 after IPT. The human ALB and HSA double-positive hepatocytes exhibited a scattered distribution of small clusters or single cells at week 15 after IPT (A, B), and only a few double-positive single cells were scattered throughout the liver lobules at week 20 (C, D). Some of the positive cells are indicated with black arrows. Images A, C, and E were stained with ALB. Images B, D, and F were stained with HSA. Images E and F show the normal pig liver tissue (negative control). Scale bars, 20 μm (A-D) and 50 μm (E, F).

Fig. 7. The results of ELISA and qPCR. (A) The concentration of human ALB in serum of FHF animals throughout the experimental period. (B) The results of qPCR for human hepatic genes in pig liver. The amount of each messenger RNA (mRNA) is shown as the fold change compared with the amount in normal human liver tissue. GAPDH was used as an internal control. The results are representative of three independent experiments.
However, no studies have investigated human BMSC transplantation in the treatment of FHF in a clinical trial or in large animal models, such as pigs. Therefore, it is important to evaluate the effects of hBMSC transplantation to clarify the precise mechanisms of their participation in liver regeneration. The cell number, transplantation time, and delivery route may influence the ultimate effectiveness of hBMSC transplantation for the treatment of FHF. Based on the doses of cells reported in three recent studies, between $2 \times 10^7$ and $5 \times 10^7$ cells are typically used to treat liver cirrhosis and end-stage hepatic failure caused by hepatitis B virus and hepatitis C virus (Amer et al., $2 \times 10^7$ bone marrow-derived hepatocyte-like cells; Kharaziha et al., $3-5 \times 10^7$ hBMSCs; Peng et al., $3.4 \pm 3.8 \times 10^7$ human bone marrow–derived mononuclear cells). In our study, $3 \times 10^7$ purified hBMSCs that possessed the classic hBMSCs phenotype and multipotential characteristics were chosen for IPT in the treatment of FHF. The subsequent follow-up showed that 13 of 15 FHF pigs that received a transplantation of $3 \times 10^7$ cells through the intraportal route survived for at least 6 months. In contrast, all 15 of the animals treated with D-gal that received a sham IPT without cells died within 4 days of FHF. Moreover, all of the animals that received the same number of cells through the peripheral vein died within 4 days. This result was confirmed by biochemical analysis, which showed that the animals in the IPT group demonstrated significantly improved liver function during the initial 4 days of cell transplantation compared with the control and PVT groups. These results indicate that $3 \times 10^7$ purified hBMSCs are sufficient to prevent death from FHF in pigs (approximately 10 kg) and that IPT is a suitable delivery approach for hBMSCs to reach the injury site and promote hepatocyte differentiation.

**Mechanism of Action of Intraportally Transplanted hBMSCs in FHF Liver Regeneration.** The contribution of BMSCs to liver regeneration via spontaneous transdifferentiation or cell fusion has been widely demonstrated in animals and humans. Recently, Chamberlain et al. demonstrated that hBMSC-derived hepatocytes exhibited widespread distribution in the liver parenchyma 56-70 days after hBMSC intrahepatic transplantation into fetal sheep. However, other investigators demonstrated that hepatocyte replacement after bone marrow transplantation occurred at a low frequency and that hBMSC-derived hepatocytes were only rarely detected 4 weeks after transplantation in a model of acute liver injury with hBMSC transfection. In our study, all 13 surviving animals exhibited a nearly normal liver structure at week 3 after hBMSC IPT. Approximately 30% of the transplanted hBMSC-derived hepatocytes were widely distributed in the repopulated liver, as demonstrated by immunohistochemistry and validated by ELISA and qPCR at weeks 2, 5, and 10 after IPT. Although the FHF animals completely recovered, the number of hBMSC-derived hepatocytes decreased to undetectable levels by week 20, which may have been the result of the natural death of the transplanted human-derived hepatocytes in recipient animals (the average life span of hepatocytes is 5 months). These results indicate that transplanted hBMSCs play a significant role in repopulating the liver in several types of damage in FHF. To augment the function of the damaged recipient liver, the transplanted hBMSCs may quickly home to the toxic, proapoptotic/necrotic liver and participate in liver regeneration via proliferation and transdifferentiation into hepatocytes, and they may stimulate the regeneration of endogenous hepatocytes via secreted molecules. We could not unequivocally demonstrate that sufficient human hepatocytes were generated from hBMSCs to significantly support the liver function and rescue the FHF animals during the initial 4 days. These issues and the possibility of paracrine, anti-inflammatory and antiapoptotic effects of transplanted hBMSCs remain to be resolved.

In conclusion, the safety, effectiveness, and mechanism of action of intraportal-transplanted human BMSCs were demonstrated for the first time in a large animal (pig) model of FHF. The results suggest that immediate IPT of hBMSCs is a safe and effective treatment for FHF and that this method can possibly be used in future clinical therapy.

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