Intraportal mesenchymal stem cell transplantation prevents acute liver failure through promoting cell proliferation and inhibiting apoptosis

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BACKGROUND: **Transplantation of mesenchymal stem cells (MSCs) has been regarded as a potential treatment for acute liver failure (ALF), but the optimal route was unknown. The present study aimed to explore the most effective MSCs transplantation route in a swine ALF model.**

METHODS: **The swine ALF model induced by intravenous injection of D-Gal was treated by the transplantation of swine MSCs through four routes including intraportal injection (InP group), hepatic intra-arterial injection (AH group), peripheral intravenous injection (PV group) and intrahepatic injection (IH group). The living conditions and survival time were recorded. Blood samples before and after MSCs transplantation were collected for the analysis of hepatic function. The histology of liver injury was interpreted and scored in terminal samples. Hepatic apoptosis was detected by TUNEL assay. Apoptosis and proliferation related protein expressions including cleaved caspase-3, survivin, AKT, phospho-AKT (Ser473), ERK and phospho-ERK (Tyr204) were analyzed by Western blotting.**

RESULTS: **The average survival time of each group was 10.7±1.6 days (InP), 6.0±0.9 days (AH), 4.7±1.4 days (PV), 4.3±0.8 days (IH), respectively, when compared with the average survival time of 3.8±0.8 days in the D-Gal group. The survival rates between the InP group and D-Gal group revealed a statistically significant difference (P<0.01). Pathological and biochemical analysis showed that liver damage was the worst in the D-Gal group, while less injury in the InP group. Histopathological scores revealed a significant decrease in the InP group (3.17±1.04, P<0.01) and AH group (8.17±0.76, P<0.05) as compared with that in the D-Gal group (11.50±1.32). The apoptosis rate in the InP group (25.0%±3.4%, P<0.01) and AH group (40.5%±1.0%, P<0.05) was lower than that in the D-Gal group (70.6%±8.5%). The expression of active caspase-3 was inhibited, while the expression of survivin, AKT, phospho-AKT (Ser473), ERK and phospho-ERK (Tyr204) was elevated in the InP group.**

CONCLUSIONS: **Intraportal injection was superior to other pathways for MSC transplantation. Intraportal MSC transplantation could improve liver function, inhibit apoptosis and prolong the survival time of swine with ALF. The transplanted MSCs may participate in liver regeneration via promoting cell proliferation and suppressing apoptosis during the initial stage of ALF.**

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KEY WORDS: **mesenchymal stem cells; stem cell transplantation; acute liver failure; apoptosis; regeneration**

Introduction

Liver damage caused by viruses, drugs, toxins or alcohol could lead to acute liver failure (ALF) with indications of hepatic encephalopathy, hepatorenal syndrome, severe infection, multiple organ failure, and even death.[1] The key strategy for the treatment of ALF is to reduce hepatocyte necrosis and stimulate hepatocyte regeneration. Current therapies including drug therapy and artificial liver therapy may reduce mortality, but the therapeutic efficacy is still limited.[2, 3] Though liver transplantation is the most effective treatment for ALF, the difficulties including severe donor shortage, numerous complications, immune rejection, requirements of immunosuppressive agents and high medical costs greatly

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limit the clinical application of liver transplantation.[4]

Stem cell transplantation is a new way in recent years for ALF treatment due to its sufficient source, low immunogenicity and the potential to differentiate into hepatocyte-like cells.[5, 6] Mesenchymal stem cells (MSCs) have the potential to differentiate into hepatocyte-like cells *in vitro* and *in vivo* with partial hepatic functions under appropriate environmental conditions.[7-11] MSCs can be regarded as the seeding cells for transplantation in relation to liver diseases.[12] Though autologous cell transplantation may prevent immunological rejection, it still has some problems in its application.

The mechanism of ALF involves various inflammatory factors and cytokines, and cellular proteins such as the Fas family and caspase signal activated apoptosis of hepatocytes.[7, 13-16] It has been indicated that the therapeutic efficacy of MSC transplantation is not only associated with the purity of MSCs, but also with the administration routes.[17] However, preclinical studies in large animal models for identifying the most effective and practical administration of MSCs are not characterized sufficiently.[18-20] Most studies are focused on sole route, thus, it is hard to compare and identify which route is the most effective and practical, and which route may be attributed to future clinical application of MSCs.

In this study, a D-Gal induced swine ALF model was established to explore the therapeutic efficacy of MSC transplantation during the treatment of ALF. In particular, the therapeutic efficacy of different MSC transplantation routes such as peripheral vein transplantation, intraportal transplantation, arteria hepatica transplantation and intrahepatic transplantation in D-Gal induced ALF was compared before and after bone MSC transplantation. The therapeutic outcome in the present study might contribute to the future clinical application of MSCs.

Methods

Animals

Chinese experimental miniature swine (15±3 kg, aged approximately 5 to 8 months) were obtained from the Laboratory Animal Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School and maintained under conventional conditions. All animal experimental procedures were approved by the Animal Care Ethic Committee of Nanjing Drum Tower Hospital.

Isolation, culture and characterization of MSCs

MSCs were isolated and cultured according to the previous report.[21] In brief, porcine MSCs were isolated by bone marrow aspirates from the iliac crests of the animals. MSCs were collected by density gradient centrifugation over a Ficoll histopaque layer (20 minutes, 400 g, density 1.077 g/mL) (TBD, China) and cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM-LG; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Gibco). The non-adherent cells were removed after the first 24 hours and changed every 3-4 days thereafter. When the cells reached up to 80% confluence, the cells were detached using 2.5 g/L Trypsin-EDTA (Gibco) and re-plated at a density of 1×10^4 cells/cm^2 for expansion. Surface markers of the cultured MSCs were identified by flow cytometric analysis (FACScan, Becton Dickinson, Franklin Lakes, NJ, USA) using fluorescein isothiocyanate (FITC)-labeled monoclonal antibody for staining to CD45 (Antigenix America, Huntington Station, NY, USA) and phycoerythrin (PE)-conjugated antibodies against CD29 (VMRD, Pullman, WA, USA), CD44 and CD90 (Becton Dickinson). Isotypic antibodies served as controls.

Swine ALF model establishment and treatment

Under general anesthesia with mechanical ventilation via an endotracheal tube, animals received a single intravenous injection of 0.3 g/kg D-Gal (Sigma, St. Louis, MO, USA) dissolved in 0.9% saline solution, via the external jugular vein.[11] Thirty-six swine were randomly divided into 6 groups including normal control group, D-Gal group, peripheral vein MSC transplantation (PV) group, intraportal MSC transplantation (InP) group, arteria hepatica MSC transplantation (AH) group and intrahepatic MSC transplantation (IH) group. D-Gal group was administrated with 0.3 g/kg D-Gal, and normal control group was administrated with the same volume saline. All groups were administrated with laparotomy except normal control group. Liver injured animals in the PV group were subjected to slow administration of 1×10^7 MSCs suspended in 2 mL normal saline via the external ear vein after D-Gal induction for 24 hours. The abdomens of liver injured animals in the InP and AH groups were opened to expose the portal vein and arteria hepatica, respectively, and approximately 1×10^7 MSCs suspended in 2 mL normal saline were slowly injected into the portal vein and arteria hepatica, respectively, after D-Gal induction for 24 hours. Liver injured animals from the IH group were opened to expose the liver and approximately 1×10^7 MSCs suspended in 2 mL normal saline were slowly injected into the liver directly after D-Gal induction for 24 hours. A 30-gauge needle was used for the procedure. The pinhole at the injection site was pressed for hemostasis. Thereafter, the laparotomy incision was enclosed in layers.