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Effect of umbilical cord mesenchymal stem cells on treatment of severe acute pancreatitis in rats

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Abstract

Background aims. The aim of this study was to investigate the effect of umbilical cord mesenchymal stem cells (UCMSCs) on severe acute pancreatitis (SAP) in rats. *Methods.* SAP was established in rats by retrograde pancreatic duct injection of sodium taurocholate. In one group, 5×10^6 cells/kg of UCMSC suspension was injected into the tail vein 0 h, 1 h, 6 h and 12 h after the induction of SAP. In other groups, different doses of UCMSC suspension (5×10^4 cells/kg, 5×10^5 cells/kg, 5×10^6 cells/kg or 1×10^7 cells/kg) were administered at 1 h. Serum amylase was assayed at 12 h. Mortality, ascites, serum tumor necrosis factor-α, interferon-γ (assayed using enzyme-linked immunosorbent assay) and the wet-dry weight of the pancreas gland were assessed at 48 h. Pathologic changes of pancreatic and pulmonary tissues were observed. *Results.* Mortality in rats receiving 5×10^6 cells/kg of UCMSCs at 0 h was 10% compared with 58% in the SAP control group. Ascites, serum amylase and wet-dry pancreatic weight significantly decreased, and production of tumor necrosis factor-α and interferon-γ were reduced. Pathologic injuries of pancreatic and pulmonary tissues were markedly alleviated. Administration of UCMSCs (5×10^5 cells/kg, 5×10^6 cells/kg or 1×10^7 cells/kg) at 1 h or 5×10^6 cells/kg at 6 h significantly reduced the severity of SAP. The effect was less marked at 12 h and with lower concentrations of UCMSCs. *Conclusions.* UCMSCs significantly decreased pancreatic injury caused by SAP in a time-dependent and dose-dependent way.

Key Words: *cell therapy, SAP, UCMSCs*

Introduction

Most patients with acute pancreatitis have mild acute pancreatitis that resolves spontaneously. However, one in five patients with acute pancreatitis develops severe acute pancreatitis (SAP), which is associated with a mortality of almost 20% (1). The implementation of early supportive treatment for respiratory and circulatory complications and for kidney failure that can accompany SAP has increased significantly over the last decade, but mortality has not markedly decreased despite this supportive treatment (2,3).

It is thought that the early pathologic changes associated with pancreatitis occur in pancreatic acinus resulting in abnormal activation of pancreatic enzymes, local congestion, edema and exudative necrosis. Other organs are affected by numerous inflammatory factors generated from the immune inflammatory reaction. This immune inflammatory reaction can have a critical impact on the circulatory, respiratory, urinary and nervous systems. Numerous drugs have been studied in an attempt to prevent the development of SAP, but to date no satisfactory treatment has been developed (4).

Mesenchymal stem cells (MSCs) have low immunogenicity and have been used in xenotransplantation. MSCs have the potential to differentiate into many different cell types and have the capacity to repair injured tissue. They have been used to treat many conditions, including pulmonary injury, acute kidney failure and myocardial infarction (5,6). It has also been reported that MSCs can act as immunoregulators, which can inhibit inflammatory injury (7) in conditions such as systemic sclerosis, Crohn disease, diabetes and systemic lupus erythematosus (8).

Multi-organ failure associated with SAP is primarily caused by over-reactivity of the immune system. The immunosuppressant effect of MSCs in SAP has not yet been investigated. In this study, we evaluated the effects of umbilical cord mesenchymal stem cells (UCMSCs) on a rat model of SAP to provide a foundation for using this intervention in clinical practice.

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Methods

Source and identification of UCMSCs

UCMSCs were provided by the State Stem Cell Industry Base (Tianjin, China). The umbilical cords were obtained from healthy mothers (approved by The Fourth Military Medical University institutional review board) during routine elective cesarean section births. Mothers fulfilled the donor selection guidelines of the Chinese Red Cross. Human umbilical cords were cut into pieces (1 mm × 1 mm × 1 mm) and were digested with 1% collagenase (30 min, 37°C) and 0.125% trypsin (30 min, 37°C). The cells were mixed with 15 mL of complete growth medium (1 mL of cells mixed with 15 mL of complete growth medium): Dulbecco's modified Eagle medium (DMEM-LG/F12; Sigma, St Louis, MO, USA), 5% fetal calf serum (Gibco-BRL, Gaithersburg, MD, USA). The cells were cultured in 15 mL of DMEM (37°C, 5% carbon dioxide, humidified incubator). When the cells were cultured 3–4 days, the DMEM was removed. After 10–14 days in 100-mm dishes, the cells were about 80–90% confluent and harvested with 0.25% trypsin and 1 mM ethylenediamine tetraacetic acid (Gibco-BRL), suspended at 1×10^6 cells/mL in 10% dimethyl sulfoxide and 40% fetal bovine serum and frozen in 1-mL aliquots in liquid nitrogen. Cells characterized by their morphology and differentiation capacity were identified using stem cell-specific markers (CD19, CD34, CD11b, CD73, CD90, CD45, CD105, HLA-DR). The procedures adopted were in accordance with the requirement of Good Manufacturing Practices. The use of cord blood for this research was approved by The Fourth Military Medical University institutional review board.

Model establishment

Male Sprague-Dawley rats (250–280 g) were provided by the Animal Experimental Center of The Fourth Military Medical University (Xi'an, China) and were housed at 25°C in a 12-h light/12-h dark cycle. Rats were fasted for 12 h before experiments and were randomly divided into groups. As described previously (9), the SAP model was induced by retrograde pancreatic duct injection of 1 mL/kg of 5% sodium taurocholate (Sigma).

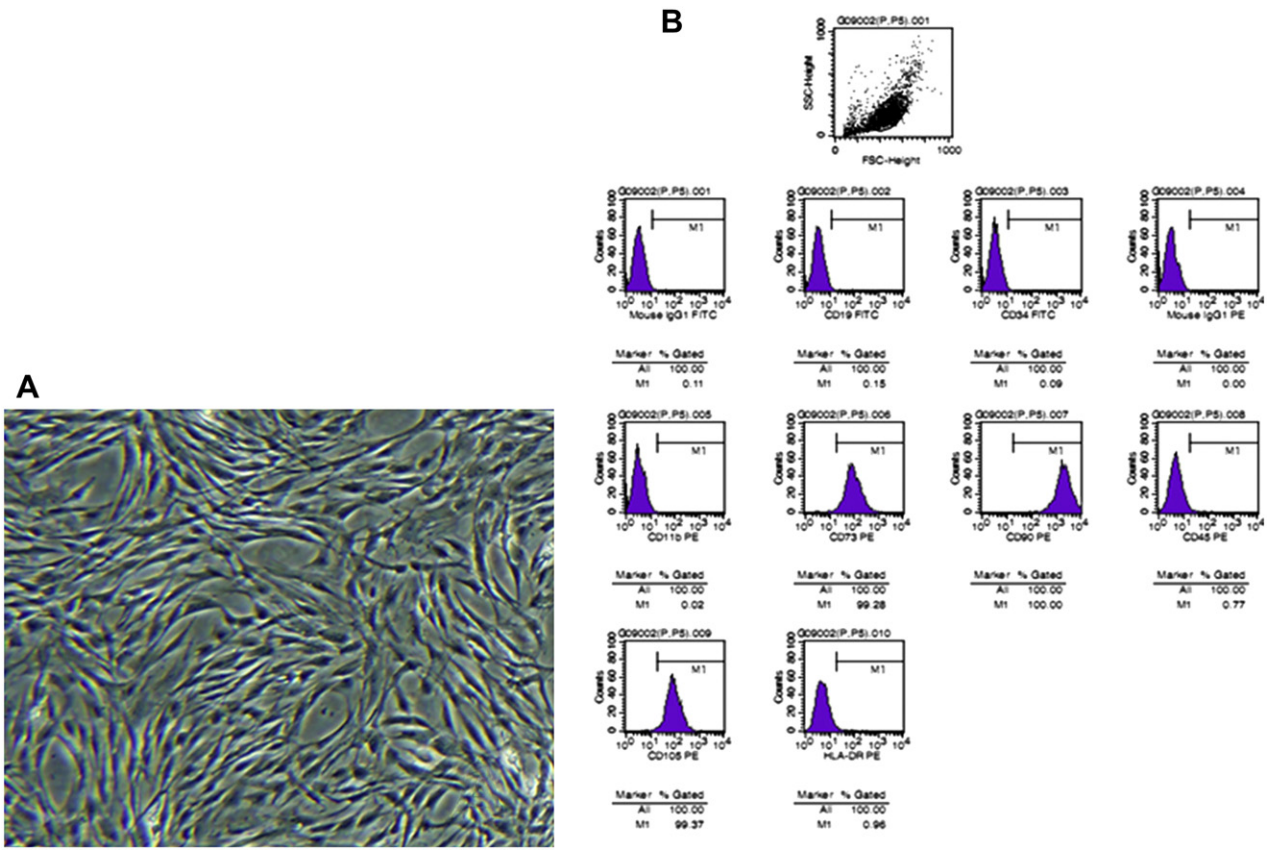

Figure 1. Characteristics of UCMSCs. (A) Size of UCMSCs was uniform; the fusiform or stellate cells had epithelioid morphology (200×). (B) Markers on the surface of MSCs were detected using flow cytometry.