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• BASIC RESEARCH •

Protective effects of transplanted and mobilized bone marrow stem cells on mice with severe acute pancreatitis

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Abstract

AIM: To evaluate the protective effects of transplanted and mobilized bone marrow stem cells (BMSCs) on mice with severe acute pancreatitis (SAP) and to probe into their possible mechanisms.

METHODS: A mouse model of SAP induced by intraperitoneal injections of L-arginine was employed in the present study. Two hundred female Balb/c mice weighing 18-22 g were randomly assigned into 4 groups. Group A was the stem cell mobilized group treated by injection of granulocyte-colony stimulating factor (G-CSF) into mice for 4 days at a dose of 40 μg· kg^{-1}· d^{-1} before induction of SAP. Group B was the group of BMSCs transplantation, in which the mice were given the isolated BMSCs via the tail vein 4 days prior to induction of SAP. Group C served as the model control and only SAP was induced. The mice without induction of SAP in group D acted as the normal control. At the time of animal sacrifice at 24, 48 and 72 h after induction of SAP, blood samples were obtained and prepared to detect serum amylase, while the abdominal viscera were examined both grossly and microscopically for the observation of pathological changes.

RESULTS: The mortality of mice in the model control, groups A and B was 34 %, 8 % and 10 % respectively within 72 h after induction of SAP. The serum level of amylase in the model control was significantly increased at all time points after induction of SAP as compared with that of the normal control (P<0.05-0.01). When the mice were pretreated with BMSCs' transplantation or G-CSF injection, their serum level of amylase was significantly reduced at 48 h and 72 h after induction of SAP in comparison with that of the model control (P<0.05-0.01). In accordance with these observations, both gross and microscopic examinations revealed that the pathological changes of SAP in mice pretreated with BMSCs transplantation or G-CSF injection were considerably attenuated as compared with those in the model control at all observed time points.

CONCLUSION: Both transplanted allogenic and mobilized autologous BMSCs can protect mouse pancreas from severe damage in the process of SAP.

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INTRODUCTION

Severe acute pancreatitis (SAP) is a life-threatening disease with a mortality rate of 20 to 30 percent[1,2]. Despite recent improvements in our understanding of the disease process and the development of a range of supportive measures, today's treatment approaches for SAP are still less than ideal. It has been demonstrated recently that multipotent somatic stem cells in adult bone marrow can exhibit tremendous functional plasticity[3-9] and reprogram in a new environmental tissue niche to give rise to cell lineages specific for the new organ site. Stem cells from bone marrow, autologous or allogenic, have been used to treat myocardial infarction[10-13], hepatic disease[14-20], nervous system dysfunction[21-25] and severe autoimmune diseases[26, 27]. However, there have been fewer reports concerned about the treatment of SAP with BMSCs as yet. Since the two critical determinants, tissue damage and higher level of pluripotent cells, seem to be the prerequisite for the transdifferentiation of transplanted BMSCs and G-CSF has been proved to have a great potency in mobilizing both hematopoietic stem cell (HSC) and mesenchymal stem cells (MSCs) of bone marrow, we hypothesized that the transplanted allogenic BMSCs, as well as the autologous BMSCs mobilized by G-CSF would exert a protective role in the treatment of SAP. The present study is therefore designed to verify our hypothesis in attempt to develop new protocols for the improvement of SAP therapy.

MATERIALS AND METHODS

Animals and experimental protocol

Two hundred female Balb/c mice weighing 18-22 g were randomly assigned into 4 groups according to different treatment protocols with 50 mice each. Group A was the stem cell mobilized group treated by injection of sc G-CSF into mice for 4 days at a dose of 40 μg· kg^{-1}· d^{-1} before induction of SAP. Group B was the group of BMSCs transplantation, in which the mice were given BMSCs isolated from male mice bone marrow at a dose of 2×10^7 per mouse via tail vein 4 days prior to induction of SAP. Group C served as the model control and only SAP was induced. The mice in group D acted as the normal control treated only with an equal amount of saline as sc G-CSF in group A and without induction of SAP.

Mouse model of SAP was prepared in all animals except that in group D according to the scheme described elsewhere[28]. Briefly, the mice were fasted overnight but allowed to free access to water. The SAP inducer, a 20 g/L of L-arginine (Sigma) solution, was freshly prepared with saline just prior to use. In induction of SAP, the animals were injected intraperitoneally L-arginine solution at a dose of 2 g· kg^{-1} twice at an interval of 1 h.

The mice in groups A, B and C were sacrificed at 24, 48 and 72 h after induction of SAP. In the meanwhile, the mice in group D were killed at the corresponding time points. At the time of animal sacrifice, blood samples were obtained and prepared to

detect serum amylase, while the abdominal viscera were examined both grossly and microscopically for the observation of pathological changes.

Transplantation of primary BMSCs

Primary BMSCs to be transplanted to group B animals were isolated from donor male mice. The whole bone marrow cells were harvested by rinsing the thighbone and shankbone' s medullary cavities with cold DMEM (Gibco, Grand Island, NY) and then fractionated by density gradient centrifugation with lymphocytic separating medium. Mononuclear component, the constituent rich in BMSCs, was obtained from the interface after centrifugation at 2 500 r/min for 30 min. After repeatedly washed in cool D-Hanks solution, BMSCs were resuspended and adjusted to a cell density of 10^8/mL with the same solution. The transplantation of primary BMSCs into group B animals was performed via the tail vein injection at a dose of 0.2 ml cell suspension per mouse 4 days prior to induction of SAP.

The transplanted stem cells were identified at the end of the experiment (72 h post SAP induction) by examining the existence of Y chromosome Sry region in recipient female mice with a PCR scheme. Briefly, the female recipients were killed by cervical dislocation and DNA samples were extracted respectively from the pancreas, bone marrow, liver and spleen. The sequence of the sense primer was 5' -ATTTATGGTG TGGTCCCG-3' and that of the antisense primer was 5' -GCTGTAAAATGCCACTCC-3' . PCR consisted of an initial denaturation step at 96 ℃ for 6 min, followed by 35 cycles at 94 ℃ for 1 min, at 52 ℃ for 1 min and at 72 ℃ for 1 min each, and a final extension at 72 ℃ for 10 min. The resulting products were analyzed by electrophoresis on a 2 % agarose gel and stained with ethidium bromide. The expected size of amplified DNA fragment was 239 base pair.

Statistical analysis

All the data were expressed as $\bar{x}\pm s$. Comparisons between the means of different groups were performed using analysis of variance followed by Student' s *t*-test. $P<0.05$ was selected to be the level of statistical significance.

RESULTS

Animal mortality

The mortality of mice in the model control was 34 % within 72 h after induction of SAP, which was considerably decreased in the mices pretreated with BMSCs' transplantation or sc G-CSF injection. The mortality of mice in groups A and B was 8 % and 10 % respectively.

Table 1 Changes of serum amylase activity after SAP induction ($\bar{x}\pm s$, n=10)

Group	Serum amylase activity (μkat/L)		
	24 h	48 h	72 h
A	25.3±4.8	40.8±7.4[b,c]	27.6±6.2[a,c]
B	25.9±4.9	40.7±6.7[b,c]	27.3±6.3[a,c]
C	46.1±12.8[a]	78.4±15.8[b]	50.2±13.3[a]
D	17.2±6.2	16.9±2.7	19.0±3.4

[a]$P<0.05$, [b]$P<0.01$ *vs* group D; [c]$P<0.05$ *vs* group C.

Alterations of serum amylase

The serum level of amylase was significantly increased in the mice of model control at all time points after induction of SAP as compared with that of the normal control ($P<0.05$-0.01). When the mice were pretreated with BMSCs transplantation or G-CSF injection, their serum level of amylase was significantly reduced at 48 h and 72 h after induction of SAP as compared with that of the model control ($P<0.05$-0.01), although the amylase value was still significantly higher than that of the normal control ($P<0.05$, Table 1).

Pathological changes

Grossly, a typical appearance of SAP changes was observed in animals of the model control. These pathological changes were progressively aggravated after SAP induction and manifested in a time- dependent manner. Twenty-four hours after L-arginine injection, the pancreas was sprinkled with hemorrhagic spots and focal necrosis with a dark-color appearance. At the same time, a little bloody ascites was noted in the abdominal cavity. At 48 h, different sizes of more hemorrhagic and necrotic focus appeared on the pancreatic surface with the increment of bloody ascites. Seventy-two hours after induction SAP, the pancreatic necrosis and blood ascites were even more prominent with saponification of fatty tissue around. Massive necrosis of multiple organs such as the intestine, lungs and kidneys was found in the mice died from SAP (Figure 1).

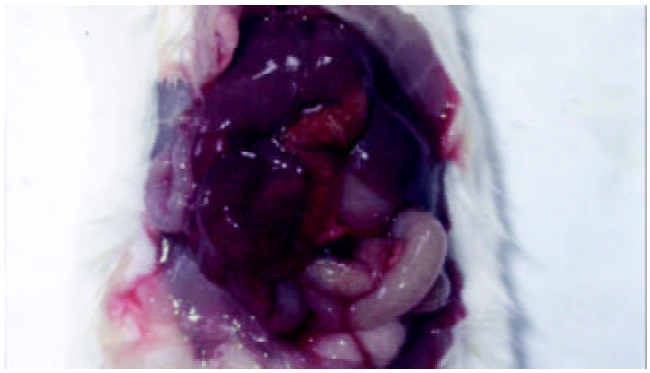

Figure 1 Gross appearance of abdominal cavity in SAP model control.

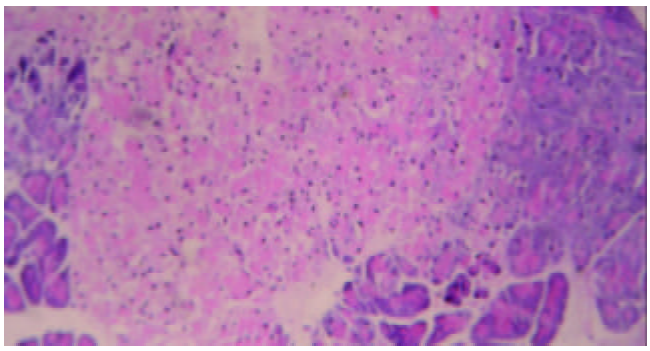

Figure 2 Pathological changes of pancreas at 24 h post SAP induction in mice of model control (HE, ×200).

Attenuated pathological lesions were noted both in group A and in group B mice, which were manifested mainly as minor pancreatic edema and congestion with less bloody or non-bloody ascites generated, no fat saponification and necrosis were observed.

In the microscopic examination, various degrees of pancreatic impairments were found in all mice injected with L-arginine. In mice of the model control, pancreatic congestion, interstitial edema, disorganized lobular architecture, as well as the focal hemorrhage and necrosis appeared at 24 h after induction of SAP, along with an obvious mesenchymal infiltration by inflammatory cells (Figure 2). The changes above were even more aggravated at 48 h and large areas of

coagulation necrosis occurred in the pancreatic parenchyma acompanied by the destroyed lobular architecture and massive inflammatory cell infiltration at the time of 72 h after induction of SAP (Figure 3).

In contrast, these pathological changes were obviously attenuated in mice pretreated with BMSCs' transplantation or G-CSF injection (Figures 4, 5). Most lobular architecture in these animals remained recognizable with alleviated hemorrhage, necrosis and infiltration of inflammatory cells, whereas some focal necrosis remained in the periphery of pancreas.

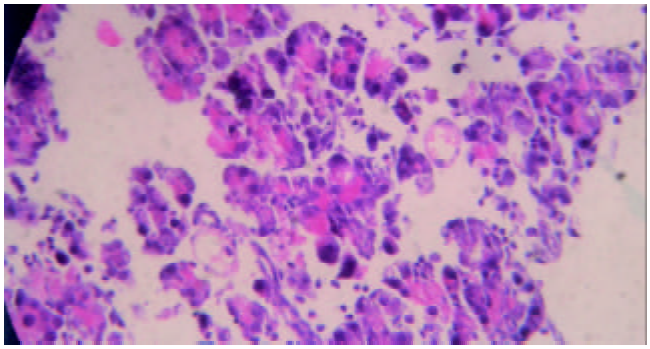

Figure 3 Pathological changes of pancreas at 72 h post SAP induction in mice of model control (HE, ×200).

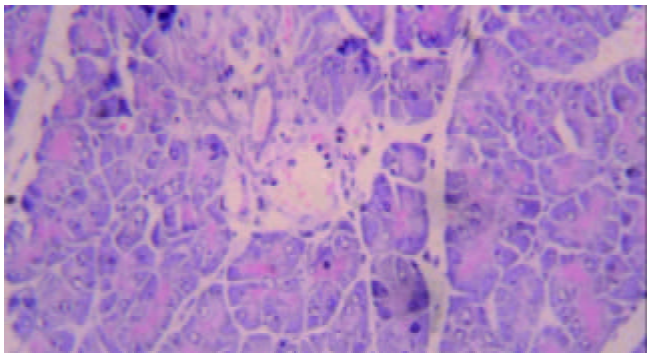

Figure 4 Microscopic changes of pancreas at 72 h after induction of SAP in mice pretreated with G-CSF (HE, ×200).

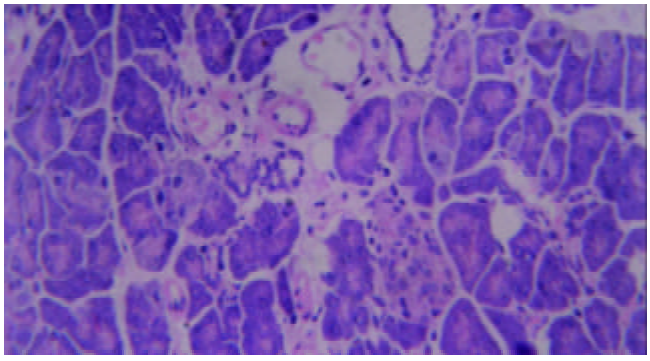

Figure 5 Microscopic changes of pancreas at 72 h post SAP induction in mice pretreated with BMSCs transplantation (HE, ×200).

Identification of transplanted BMSC

All sampled tissues from the pancreas, liver, spleen and bone marrow of the female recipients were demonstrated harboring a DNA fragment, 239 bp in length, of Y chromosome Sry region of donor mice, indicating that the engrafted BMSCs could migrate and survive in the impaired pancreas of SAP animals (Figure 6).

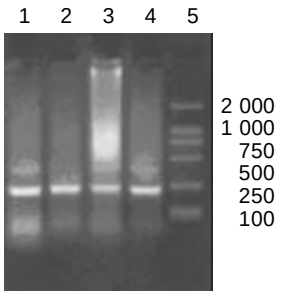

Figure 6 PCR products of Y chromosome Sry region gene existed in some organs of recipient female mice after male BMSCs transplantation demonstrated by agarose gel electrophoresis. Lane 1: pancreas, Lane 2: liver, Lane 3: liver, Lane 4: spleen, Lane 5: DNA marker.

DISCUSSION

The management of SAP has changed significantly over the past 20 years. In contrast to surgical intervention, there is now a strong tendency towards a more conservative therapy. The established treatment of SAP included aggressive fluid resuscitation, oxygen supplementation, prophylactic use of antibiotics, enteral feeding and intensive care support of any failing organ or system[29]. Nevertheless, the fact that these therapeutic modalities did not aim directly at the etiological factors made the treatment lack of specificity. Therefore, it remains a great challenge for the improvement of SAP therapy in the daily clinical practice. Recently, it has been shown that somatic stem cells were capable of regenerating injured tissue and improving the functions of involved organs[6,30-32], which might be used also as a new potential therapeutic modality for SAP treatment.

Bone marrow is an ideal source of stem cells. The multidirectional differentiation potential of BMSCs has been proved to be more than what we expected[32]. It has been reported that BMSCs could transdifferentiate into a variety of cell types derived from three embryonic layers such as endoblast-derived hepatic cells and lung alveolar epithelial cells, mesoblast-derived kidney and muscle cells, and ectoblast-derived neurons and epidermic cells. Furthermore, some authors have shown that bone marrow might serve as an extra-pancreatic hideout for the pancreatic stem cells[33,34] that contributed to the adult islet neogenesis. Transplantation with bone marrow cells has been used in several animal experiments for the treatment of types I and II diabetes mellitus, which has been achieved some promising results. All these facts indicate that BMSCs harbor a biological basis which can be used as an excellent candidate for SAP therapy.

In the present study, we observed the protective effects of BMSCs transplantation in a mouse model of SAP. It showed that a considerably reduced mortality and serum amylase activity, as well as obviously attenuated pancreatic pathological changes in the animals treated by transplantation of BMSCs or by injection of G-CSF, which presented a striking contrast to those of the model control. Regarding the mechanisms involved, these protective effects might be mediated by the rehabilitative action of BMSCs, which was partially supported by the existence of transplanted stem cells in the assaulted pancreas with a PCR scheme. The transplanted BMSCs might be ' tweaked' from the peripheral blood circulation to the injured tissues whenever SAP took place, where they exerted the function of pancreatic stem cells to regenerate the destroyed cells. As a result, gradual necrosis of impaired pancreas was effectively prevented. On the other hand, critical ill conditions such as SAP can usually caused serious injuries to multiple organs or systems, leading to a poor capability for the tissue repair. At

this time, transplanted stem cells in peripheral circulation were no doubt helpful for regeneration of injured tissues[38]. However, the direct evidence of BMSCs transdifferentiating to pancreatic cells should be further demonstrated by *in situ* hybridization and immuneohistochemistry.

An important phenomenon that deserved to note in the present study was the protective effects of autologous BMSCs mobilized by G-CSF injection in this model of SAP, whose efficiency was similar to that of alligeneic BMSCs transplantation. It implies that BMSCs, no matter autologous or allogenic, could get to and reside in the impaired tissue via blood circulation to repair the dysfunctional organs. Recently, Jensen and his associates[31] have shown that a normal physiological process of tissue regeneration and repair could be achieved by *in situ* mobilization of autologous stem cells from the bone marrow. Through the stimulation of normal stem cell migration, therapeutic benefits could be achieved with less invasive regimens than the removal and re-injection of stem cells. In this way, some obstacles in the allograft such as rejection and shortness of tissue donor supply would be also overcome easily. However, there were fewer BMSCs with weaker expansion and differentiation capacity in the peripheral circulation. Once mobilized by G-CSF, BMSCs in the peripheral circulation were estimated to be increased about 250 times higher than the baseline, along with an enhanced expansion and differentiation capacity. Thereby, autologous BMSCs transplantation could be performed safely and conveniently. Further research should be focused on the more efficient mobilization of autologous BMSCs to attract them to the injured region for tissue repair. This cell-restoring therapy may serve as a new modality in the future management of SAP and other serious diseases.

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