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Mesenchymal stem cells increase antioxidant capacity in intestinal ischemia/reperfusion damage☆

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) may have beneficial effects in reversing intestinal damage resulting from circulatory disorders. The hypothesis of this study is that MSCs increase antioxidant capacity of small bowel tissue following intestinal ischemia reperfusion (I/R) damage.

Methods: A total of 100 rats were used for the control group and three experimental groups, as follows: the sham control, local MSC, and systemic MSC groups. Each group consisted of 10 animals on days 1, 4, and 7 of the experiment. Ischemia was established by clamping the superior mesenteric artery (SMA) for 45 min; following this, reperfusion was carried out for 1, 4, and 7 days in all groups. In the local and systemic groups, MSCs were administered intravenously and locally just after the ischemia, and they were investigated after 1, 4, and 7 days. The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) activities, as well as malondialdehyde (MDA) and total protein levels, were measured.

Histopathological analysis was performed using light and electron microscopy. The indicators of proliferation from the effects of anti- and pro-inflammatory cytokines were evaluated using immunohistochemistry.

Results: MDA was increased ($P < 0.05$) in the sham control group and decreased ($P < 0.05$) in the MSC groups. SOD, CAT, and Gpx were decreased in the local MSC group ($P < 0.05$). The highest level of amelioration was observed on day 7 in the local MSC group via light and electron microscopy. It was found that the MSCs arrived at the damaged intestinal wall in the MSC groups immediately after injection. Pro-inflammatory cytokines interleukin-1β (IL1β), transforming growth factor-β1 (TGFβ1), tumor necrosis factor-α (TNFα), IL6, MIP2, and MPO decreased ($P < 0.05$), while anti-inflammatory cytokines EP3 and IL1ra increased ($p < 0.05$) in the local and systemic MSC groups. In addition, proliferation indicators, such as PCNA and KI67, increased ($P < 0.05$) in the local and systemic MSC groups.

Conclusions: Parallel to our hypothesis, MSC increases the antioxidant capacity of small bowel tissue after intestinal I/R damage. The MSCs migrated to the reperfused small intestine by homing and reduced oxidative stress via the effects of SOD, CAT, and Gpx, as well as reducing the MDA level; thus, they could increase antioxidant capacity of intestine and have a therapeutic effect on the damaged tissue. We think that this effect was achieved via scavenging of oxygen radicals, suppression of pro-inflammatory cytokines, and increasing the expression of anti-inflammatory cytokines.

Ischemia reperfusion (I/R) damage in the small intestine is a life-threatening condition that frequently arises from circulatory disorders in the gastrointestinal system involving the superior mesenteric artery (SMA), and it is associated with several factors. Such damage results in minor or severe local or extensive tissue damage, depending on the length of time over which the injury occurs [1–4]. Mesenteric circulatory disorders may emerge in intravenous cases, including arterial thrombosis, emboli, Henoch-Schönlein purpura, and disseminated intravascular coagulation, while clinical disorders, such as volvulus, invagination, incarcerated inguinal hernia, tumor, and fibrotic bands, may also arise [1]. In addition, small intestinal ischemia sometimes occurs in transplantation surgery. Hypertension, vasoconstriction, blood viscosity disorders, arteriosclerosis, hypotension, and similar cases without intravenous blockage may also lead to small intestinal ischemia [2,3].

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Due to their regenerative properties, mesenchymal stem cells (MSCs) obtained from bone marrow can be used for therapeutic purposes in most tissues [3]. For the intestine, it has been reported that stem cells derived from bone marrow migrate to the intestine and transform into stromal cells in cases of tissue damage caused by radiation [4]. Furthermore, it has been shown that the MSCs settle in the intestine and become myogenic cells during the restorative phase of colitis caused by dextran sodium sulfate [5]. It has been observed that MSC administration results in rapid recovery from acute and chronic small intestinal damage caused by pelvic and abdominal radiotherapy, suggesting their use for therapeutic purposes [6]. These properties are not limited to paracrine signal mechanisms; they also include immunomodulation in the affected environment and differentiation potential in damaged tissue [7]. It has been noted that, because of their migrating capability, stem cells can reach and repair damaged tissue [8].

Jiang et al. [3] examined the integrity of the intestinal barrier using parameters like intestinal transparency and bacterial translocation, and they concluded that MSCs infused into the submucosal area protect the intestine against I/R damage. They also suggested that MSCs suppress inflammation and increase cell proliferation after intestinal I/R injury. Furthermore, MSCs have been reported to have positive effects on some significant ultrastructural parameters in the intestine after I/R injury [9]. Overall, the data suggest that MSCs can be utilized to repair the damage caused by oxygen radicals in the intestine. However, to the best of our knowledge, the antioxidant capacity of MSCs in intestinal I/R damage has not been investigated previously. The hypothesis of this study is that MSC increases the antioxidant capacity of small bowel tissue following intestinal I/R damage.

1. Material and method

In this experimental study, 10- to 12-week-old female Sprague–Dawley rats weighing 200–250 g were used. The animals were provided by Experimental Animal Breeding and Research Unit of Trakya University, Edirne, Turkey. They were kept in standard laboratory conditions (22 ± 1 °C temperature, 55% humidity, 12-h light/dark circle) and fed with standard feed and tap water. Feeding of the animals was stopped 12 h in advance of the experiment, with only water provided. The local ethics committee approved the study protocol with approval number TUHDYEK-2011/58.

1.1. Experimental procedure

A total of 100 rats were used for one control (Group C, no I/R injury group, $n = 10$) and three experimental groups, namely the sham control group (Group ShC), systemic MSC group (Group S), and local MSC group (Group L); each group consisted of 10 animals for each of days 1, 4, and 7 of the experiment. The group protocol was as follows:

- Group C: Control group; no I/R injury, (n = 10);
- Group ShC: Sham control groups; ischemia model applied on operation day (day zero) and the rats were sacrificed after reperfusion period on days 1 (C1, n = 10), 4 (C4, n = 10), and 7 (C7, n = 10), but no treatment with MSC was administered;
- Group S: Systemic MSC group; ischemia model applied on operation day and MSCs (0.5×10^6 cells) were transplanted with systemic administration just after ischemia (on day zero) and the rats were sacrificed after reperfusion period on days 1 (S1, n = 10), 4 (S4, n = 10), and 7 (S7, n = 10); and
- Group L: Local MSC group; ischemia model applied on operation day and MSCs (0.5×10^6 cells) were transplanted with systemic administration just after ischemia (on day zero) and the rats were sacrificed after reperfusion period on days 1 (L1 n = 10), 4 (L4 n = 10), and 7 (L7 n = 10).

Animals were anesthetized using xylazine/ketamine (10/50 mg/kg intramuscularly, Eczacıbaşı, Istanbul, Turkey) for the surgical procedures. In Group C, rats were sacrificed after the anesthesia and intestine was harvested. In group ShC, each rat's abdomen was opened and the SMA was dissected and tied up for 45 min to produce ischemia. The abdomen was reopened after 45 min to initiate the reperfusion period, and the intestine was harvested after 24 h of reperfusion on day 1, 4 and 7 days. In Groups L and S, animals were reopened after 45 min and MSCs were applied and left for a reperfusion period of 24 h for day 1 4 and 7 days and intestine was harvested.

MSCs were taken from the bone marrow of Sprague–Dawley rats with phenotypic properties defined and marked using green fluorescent protein (GFP). The MSCs used in the study were cells that achieved 60–70% confluency, which were kept in dry ice or flasks and frozen in cryovials under appropriate conditions; immediately after ensuring blood flow from the SMA, submucosal transplantation was carried out of 1 × 106 cells of 1-ml volume in groups receiving local MSC administration at 10 points with equal intervals from the Treitz ligament to the cecum, and the abdomen was closed [3]. For systemic MSC administration, 1 ml of solution with 0.5 × 106 cells was systemically administered from the vena cava inferior (VCI) over 4–5 min.

All intestinal tissue samples were obtained from the ileal segment of the intestine 5 cm proximal to the ileocecal region for all parameters. Tissues were fixed for 24 h with formalin for light microscopy or 4% glutaraldehyde and 1% osmium tetra oxide for electron microscopy. They were washed with NaCl 0.9% and dried, and then they were stored in a deep freeze for freezing at −80 °C for investigation of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx) levels.

1.1.1. Biochemical parameters to be examined using tissue samples

1.1.1.1. Measuring the malondialdehyde and total protein quantities in tissue samples. The concentration of MDA was determined by a colorimetric method. Samples were homogenized with 150 mmol/L ice-cold KCl for the measurement of MDA. The MDA concentrations in the small intestinal tissue were assayed in the form of thiobarbituric acid-reacting substances. 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of acetate buffer (3 M, pH: 3.5), 1.5 mL of 0.82% thiobarbituric acid were added to the supernatant (0.25 mL). This mixture was heated to 95 °C for 45 min. After cooling with tap water, 0.5 mL of 35% sodium dodecyl sulfate was added and the mixture was heated to 95 °C for 10 min. Then it was shaken vigorously and centrifuged at 2500× rpm for 10 min at 25 °C. The absorbance of the final solution was measured at 532 nm. MDA was quantified using standard graphic and expressed as μmol/L MDA [11].

The total protein assignment in tissues was performed spectrophotometrically using the Lowry's method [12]. The values attained spectrophotometrically at 500 nm were expressed as mg/ml protein using the standard diagram prepared with the bovine serum albumin.

1.1.1.2. Determination of SOD, Gpx and CAT activities in tissue samples. A common extraction procedure was carried out for the SOD, Gpx and CAT activities in tissue samples. The tissue samples were homogenized with glass–glass homogenizer in 1:10 (*w*/*v*) phosphate buffer (containing 1% triton X-100, 0.05 M, pH: 7.0, Merck, Darmstadt, Germany). The homogenate was centrifuged at +4 °C, 10.000×*g* for 20 min, and supernatant was separated (S_1) and used for Gpx activity. 0,2 ml ethanol (1%) was added to 1 ml of the S_1 and used for CAT activity. Furthermore, 0.6 ml of ethanol-chloroform (3:5 *v*/v) was added to 2 ml of S_1, mixed inside ice for 15 min and incubated, then centrifuged at +4 °C, 10.000×*g* for 15 min and the resulting supernatant (S_2) was used for SOD activity [13].

Super oxide dismutase and Gpx enzyme activities were determined spectrophotometrically using "Ransod SD 125 (Randox, West Virginia, USA) Kit" [14,15] and "Ransel RS 505 (Randox, West Virginia, USA) Kit" [18–20] respectively. One unit of SOD activity was determined as the amount of enzyme for 50% inhibition of the, INT [2-(4-iodophenyl)-3-