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ORIGINAL ARTICLE

Trial evaluation of bone marrow derived mesenchymal stem cells (MSCs) transplantation in revival of spermatogenesis in testicular torsion

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Abstract Defective spermatogenesis due to the failure in germ cell proliferation and differentiation is the major sign of male infertility pathogenesis; and male factor is involved in up to half of all infertile couples. Testicular torsion is an acute vascular event causing the rotation of the vascular pedicle of the testis, thereby impeding the blood flow to the testis and the scrotal contents. Azoospermia caused by torsion in testis is a common source of impotency, which has not been touched by this approach, yet. Here, we use the capacity of mesenchymal stem cells (MSCs), as multipotent adult stem cells, to revive spermatogenesis in torsion-induced azoospermia. For this purpose, MSCs were extracted from rat bone marrow, cultured and transplanted into torsioned testis. Germ cell

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specific markers (Oct4, Vasa and c-Kit) were monitored for the differentiation of MSCs after transplantation into the torsion azoospermed testis. This study is a trial evaluation of mesenchymal stem cells in rat torsion testis to follow up the regenerative capacity of stem cells in spermatogenesis revival. These approaches can provide the powerful tools to investigate the basic biology of stem cells for reproductive engineering and infertility treatment.

1. Introduction

Mesenchymal stem cells (MSCs) are widespread in adult organisms and may be involved in tissue maintenance and repair, as well as in the regulation of homeostasis and immunological responses (1,2). Bone marrow derived mesenchymal stem cells (BM-MSCs) are different from other somatic stem cells in that they differentiate not only into the same mesodermal lineage but also into other lineages with different embryonic origins such as bone marrow, muscle, liver, lung, and skin (3–5).

Generation of germ cells from uncommon sources, such as mesenchymal stem cells has been of particular interest in recent years due to their significant clinical applications. Recent studies have shown the ability of both embryonic (6,7) and adult stem cells (8,9) to differentiate into primordial germ cells and further mature gametes. By day 6 after birth, primordial germ cells (PGCs), the origin of male germ cells that arise from proximal epiblast, migrate to the basement membrane of the seminiferous tubules and become spermatogonial stem cells (SSCs) (10). SSCs are responsible for maintaining the spermatogenesis process throughout the life time (11–13). A key step in investigation of male infertility is the appropriate classification of impaired spermatogenesis (14,15). Testicular torsion, the twisting of the spermatic cord, which cuts off the blood supply to the testicle and surrounding structures within the scrotum, is a true urologic emergency and must be differentiated from other complaints of testicular pain because a delay in diagnosis and management can lead to loss of the testicle (16–18). Torsion of spermatic cord is the main cause of testicular torsion that constitutes a surgical emergency affecting newborns, children, and adolescent boys. Studies have shown that between 16% and 42% of boys with acute scrotal pain have testicular torsion (19–22). Surgery is usually required and should be performed as soon as possible after symptoms begin. If surgery is performed within 6 h, most testicles can be saved (17,18).

Mesenchymal stem cells possess the capacity to trans-differentiate into epithelial cells and lineages derived from the neuro-ectoderm, and in addition, these cells can migrate to the sites of injury, inflammation, and to tumors. These properties of mesenchymal stem cells make them promising candidates for use in regenerative medicine (23,24).

This study aims to investigate the regenerative potency of CM-DiI labeled bone marrow derived-mesenchymal stem cells (BM-MSCs), implanted into the torsion rat testis. This capacity was monitored using different germ cell specific markers including Oct4, Vasa and c-Kit by immunohistochemistry analysis. This trial experiment and long time follow up studies can help to introduce novel therapeutic approaches in patients suffering testicular torsion.

2. Methods and materials

2.1. Isolation and expansion of bone marrow derived-mesenchymal stem cells

Mesenchymal stem cell derivation was done using the previously well established protocol (25,26). Briefly, MSCs were collected by flushing the femurs of 4–6 week old male rats. Cells were cultured in 25 cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Scotland) supplemented with 15% fetal bovine serum (FBS) (Gibco, Scotland), 100 µl/ml penicillin, and 100 mg/ml streptomycin (Gibco, Scotland). Non-adherent cells were eliminated by a medium change at day 2 and after confluency, the adherent cells were detached by trypsinization and replated using a 1:3 passage. MSCs expanded normally with a spindle appearance and were readily grown *in vitro* by successive cycles of trypsinization, seeding and culture, every 3 days. According to the fact that fibroblast cells will be eliminated during trypsinization in consequent passages, we considered these fibroblasts-like cells as MSCs.

2.2. CM-DiI labeling of BM-MSCs

Prior to injection, the MSCs were labeled with the fluorescent marker 1,1′-didodecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (CM-DiI) (Invitrogen™, c7000). CM-DiI is a lipophilic dye that labels cells by means of lateral diffusion through the plasma membrane, with an absorbance wavelength of 480 nm and emission at 565 nm. The labeled cells were then visualized by fluorescence microscopy (Olympus, IX70). For this purpose, 50 µg of CM-DiI was dissolved in 50 µl dimethyl sulfoxide (DMSO) as the stock solution. Twenty-four hours before injection, 2 µl of the solution was diluted in 1 ml phosphate buffered salin (PBS) to prepare a working solution. The medium was then replaced by CM-DiI solution and incubated at 37 °C for 5 min, followed with incubation at 4 °C for 10–15 min. After this time, normal medium was added to the cells and incubated at 37 °C until transplantation. Before transplantation, the cells were washed with PBS, trypsinized, counted, and injected as explained below.

2.3. Surgical process and cell transplantation

To follow up the regenerative potency of MSCs, we divided 42 male Wistar rats into two groups of control (left testis torsion/detorsion, $n = 7$), and treatment (left testis torsion/detorsion + CM-DiI labeled cells, $n = 7$) to be analyzed at three time points (2, 45 and 95 days, since we had technical limitation for using CM-DiI dye no longer than 100 days) following transplantation. In both groups, the right testis was considered as positive control. All surgical procedures were performed