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Bone marrow–derived mesenchymal stem cells ameliorate parotid injury in ovariectomized rats

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Abstract

Background aims. Parotid hypofunction causes life-disrupting effects, and there are no effective medications for xerostomia. We hypothesized that mesenchymal stem cells (MSCs) have repairing effects on parotid glands of ovariectomized (OVX) rats. *Methods.* Forty-five adult female rats were divided into three equal groups: group I (Control group), group II (OVX-group) and group III (OVX rats that received MSCs at 4 and 8 weeks post-ovariectomy). At 12 weeks post-ovariectomy, histological (Masson's trichrome and periodic acid–Schiff with alcian blue stains), immunohistochemical (caspase-3 and CD44) and morphometric studies and salivary flow rate and saliva pH determination were carried out. *Results.* Histologically, the OVX group displayed numerous irregular vacuolated acini, thickened septa with marked cellular infiltration and vascular congestion. Degenerated organelles and few or irregular secretory granules with a different density were observed. Caspase-3-positive cells were highly expressed. MSC-treated glands exhibited a considerable degree of preservation of glandular architecture with numerous CD44-expressing and few caspase-3–expressing cells. Significant decrease of the salivary flow rate in the OVX group was detected, which reverted to normal levels in group III. *Conclusions.* MSCs ameliorated the damaging effects of ovariectomy on the parotid glands.

Key Words: *Caspase-3, CD44, mesenchymal stem cells, ovariectomy, parotid*

Introduction

The salivary glands produce saliva, which contains several enzymes, mucus and antibacterial compounds including lysozyme and immunoglobulin A. It has a fundamental role in starch digestion and maintaining oral health. The ability to speak, swallow and taste food is also heavily reliant on its presence [1].

Saliva composition shows hormone-related changes during the menstrual cycle and pregnancy, suggesting that sex hormones may have a role in the control of salivary gland functions [2]. The presence of salivary gland estrogen receptors (ERs) suggests an important role for estrogen in their physiology. Sex steroids affect the development and function of salivary glands, despite the fact that these glands express low levels of steroid receptors [3–5].

Salivary hypofunction could be caused by low estrogen levels [6]. This hypofunction and related xerostomia will degrade oral and dental health, with subsequent loss of the antibacterial properties of saliva. Any dysfunction of saliva production is considered a significant clinical concern because it seriously reduces quality of life. Impaired salivary gland functions (quality and quantity) are considered common menopausal disorders [7]. In the postmenopausal period, morphological salivary gland changes have been noted [6]. Estrogen deficiency can cause oxidative stress, which triggers production of reactive oxygen species that cause cell damage [8,9].

There is a wide range of therapies in the treatment of hyposalivation and xerostomia, but their efficiency is controversial [10]. Artificial lubricants and salivary gland stimulants transiently stimulate the residual gland and hence ameliorate the hyposalivation [1,11]. Many of these treatments are pervaded wit numerous side effects, however, such as excessive sweating and tearing, flushing, voice change, tremor, nervousness, and diarrhea [12]. Thus, there is no satisfactory therapy for the deleterious effects of menopause on

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the salivary glands [13]. The development of an alternative treatment to provide an effective long-term solution is imperative.

In recent years, many researchers have diverted their attention to stem cells, tissue engineering and regenerative medicine approaches [14]. Mesenchymal stem cells (MSCs) derived from dental and non-dental sources have been effectively used for regeneration in the maxillofacial region, and a few studies in animal models have demonstrated beneficial effects of their therapy on salivary gland dysfunction [15,16].

This study aimed to examine the effects of a hypoestrogenic condition caused by ovariectomy on the parotid gland of albino rats and to evaluate the possible therapeutic role of MSCs.

Methods

Animals

The study was conducted at the Animal House of Faculty of Medicine, Zagazig University. All experimental procedures were conducted after approval from the Zagazig Animal Care Committee and conformed to the principles laid down by the National Research Council Guide for the Care and Use of Laboratory Animals.

Forty-five nulliparous female albino rats aged 8–10 weeks and weighing approximately 180–200 g were obtained from the animal house. They were kept in polypropylene cages with a temperature of 25 ± 1°C, humidity of 55 ± 5% and a 12-h light/dark cycle (light-controlled room) with *ad libitum* water and a commercial pelleted diet.

Surgical procedure

After 1 week of acclimatization, all surgeries were performed under 20-mg/kg dose of sodium thiopental (Pentothal) anesthesia. During ovariectomy, clipping was performed, and antisepsis with iodinated alcohol in the lower abdomen was done. A midline longitudinal incision was made passing through the peritoneum and abdominal muscles with a surgical blade (no. 11). At the peritoneal cavity, the adipose tissue was retracted until the fallopian tubes and ovaries were identified. Suturing of the uterine horns was performed with a single 4.0 catgut suture, allowing for bilateral resection of the ovaries. At the end of the surgical procedures, external sutures and internal were placed using 4.0 nylon, and a single 4.0 catgut absorbable thread, respectively [17].

After the incision was closed, rats were allowed to live in the appropriate environment. They were given prophylactic ampicillin (4000 IU/kg, intraperitoneal) for 3 days. For the first 2 days after operation, the rats were given metamizole sodium (25 mg/kg) as an analgesic twice a day. The wound dressing was applied every day for a week to prevent the risk of infection. These rats were maintained for 12 weeks.

Sham operation was done in the same manner, but the ovaries were simply exposed and then left intact.

Preparation of bone marrow–derived MSCs

Bone marrow was flushed out the femurs and tibiae of 10 male albino rats of 6weeks old using Caplan's method [18]. Flushing out the bone cavities was done with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine medium. Nucleated cells were isolated with a density gradient and were re-suspended in a complete culture medium supplemented with 1% penicillin- streptomycin. The cells were incubated in a 5% humidified CO2 incubator at 37°C for 2 weeks as a primary culture or upon the formation of large colonies. When large colonies developed (80–90% confluence), the cultures were washed twice with phosphate buffer saline (PBS). The cells were detached with 0.5 mL of 0.25% trypsin in 1 mM 0.02% EDTA for 5 minutes at 37°C. After centrifugation, cells will be re-suspended in PBS [18]. The tri-lineage differentiation potential of the bone marrow–derived (BM)-MSCs was not assessed.

Cell viability analysis

Cell viability was done using trypan blue dye exclusion test, which is based on the principle that live cells possess intact cell membranes that exclude the dyes, whereas dead cells do not. The viable cell will have a clear cytoplasm, whereas a nonviable cell will have a blue cytoplasm [19].

Labeling of BM-MSCs with Paul Karl Horan 26 (PKH-26) (red fluorescence cell linker)

BM-MSCs were harvested during the second passage and labeled with PKH26 dye. Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution [20].

Phenotypic characterization of the primary BM-MSCs with the flow cytometry

Flow cytometric analysis of BM-SCs was performed as previously described [21]. Briefly, BMSCs were trypsinized and cell suspensions were washed twice with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA). A total number of 1×10^5 BM-MSCs were used for each run. The cells were characterized by flow cytometry using antibody CD markers. A number of 5×10^5 cells were centrifuged and separated. Fetal bovine serum (95%; 100 μL) and PBS (5%) were added and homogenized

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