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Effect of Bone Marrow Derived-Mesenchymal Stem Cells on Submandibular Salivary Glands of Carbimazole Induced Hypothyroidism in Albino Rats

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ABSTRACT

Purpose: This study was aimed to estimate the effect of bone marrow derived mesenchymal stem cells (BM-MSCs) on the submandibular salivary gland of induced hypothyroidism in rats. **Material and Methods:** Thirty adult male albino rats weighing between 200-250 grams were used in the present study. The animals were divided into three equal groups: Group I (control group), Group II (carbimazole induced hypothyroidism group): Rats received a daily dose of carbimazole (5mg/250g/day) for 5 weeks, Group III (BM-MSCs treated group): Hypothyroidism was induced similar to group II, then rats were injected with 1x 10^7 BM-MSCs cells. All Rats were euthanized after 8 weeks (5 weeks of hypothyroidism induction + 3 weeks of stem cells injection). **Results**: group (II) showed excessive destruction and degenerative changes of the acini and ducts in comparison to group (I), while group (III) revealed marked improvement of the parenchymal elements after BM-MSCs injection. **Conclusions:** This study supposes that BM-MSCs improve salivary glands damage following induction of hypothyroidism. Therefore, BM-MSCs can be applied as cell-based therapy for restoration of hypothyroid-induced salivary hypofunction.

KEYWORDS

Mesenchymal stem cells, Salivary gland, Carbimazole, Hypothyroidism.

INTRODUCTION

Hypothyroidism is the condition in which the production of thyroid hormones declines beneath the normal level. As a consequence of impairment in mechanisms that controls the formation of thyroid hormones, thyroid dysfunction, or occurs due to complications throughout treatment of hyperthyroidism [(1)].

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Deficiency of thyroid hormones production causes serious defects that may lead to stress and depression which disturb patient's daily functions. Oral manifestations of hypothyroidism appear in the form of enlarged tongue and increased susceptibility to dental caries. Enlarged salivary glands were also noticed and serve as a useful hint in the diagnosis of hypothyroidism [2]. Submandibular glands are well known to be a target organ for thyroid hormones. A correlation had been shown between salivary gland dysfunction/Sjögren's syndrome and autoimmune thyroiditis. It was found that both caused xerostomia [3].

Stem cells are undifferentiated cells and are the building blocks of specialized tissue through their ability to differentiate into multiple cell lineages. However, their ability to self-renew makes them useful in potential therapy [4]. Stem cell therapy is a part of regenerative medicine that involves the use of undifferentiated cells in order to cure diseases. It has been investigated for the treatment of many conditions, including neurodegenerative conditions such as Parkinson's disease, cardiovascular disease, liver disease, diabetes and autoimmune diseases [5]. The main multipotent stem cell types undergoing research are haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) [4].

MSCs are self-renewable undifferentiated cells existing in bone marrow and in mesenchymal tissues. They can differentiate into osteoblasts, myocytes, chondrocytes, adipocytes, tenocytes, hemopoietic supporting stromal cells, moreover into non-mesodermal cells from skin, lung, brain, kidney, and vascular tissues [6]. MSCs have been successfully used for regeneration in maxillofacial area like regeneration of pulp, periodontal ligament, tooth, production of enamel and dentin, craniofacial regeneration, repair of cleft lip and palate, and salivary gland regeneration [5].

Moreover, bone marrow-derived mesenchymal stem cells (BM-MSCs) have been used effectively in cell therapy protocols to treat patients with idiopathic aplastic anemia, osteogenic imperfect, graft-versus-host disease and myocardial infarction [6]. Several reports have also showed that BM-MSCs played significant role in the healing of gut injuries and contribute in formation of gastrointestinal tissues [7]. It was found that BM-MSCs ameliorated acinar cell necrosis, pancreatic edema and inflammatory cell infiltration in rats with severe acute pancreatitis [8]. Moreover, previous study spotted that BM-MSCs were able to minimize radiation induced mucositis in tongue of albino rats [9]. Lastly, recent investigation revealed that wounds treated with BM-MSCs lead to acceleration of re-epithelialization, angiogenesis and granulation tissue formation in rats [10].

MATERIAL AND METHODS

1- Animals:

Thirty adult male albino rats were used in this study weighing between 200-250 grams. The rats were housed in separate cages in the animal house of Faculty of Medicine, Cairo University. The experiment was done under supervision of specialized veterinarian since their housing till getting rid of the sacrificed bodies.

The study was accepted by Ethics Committee, Faculty of Dental Medicine for Girls, Al Azhar University (REC18-082).

2- Experimental design:

After one week acclimatization period, the animals were randomly divided into three groups (ten rats each):

Group I (Control group): Rats of this group allowed accessing freely balanced diet and freshwater supply throughout the experimental period.

Group II (Carbimazole induced hypothyroidism group): For induction of hypothyroidism, rats received a single daily dose of carbimazole (5mg/250g/day) dissolved in 3 ml of distilled water, and delivered by intragastric tube for 35 successive days (5 weeks) [11].

To confirm induction of hypothyroidism, blood samples were collected five weeks after the beginning of carbimazole treatment for measurement of serum T3 and T4 levels [12].

Group III (BM-MSCs treated group): Hypothyroidism was induced similar to group II, then rats were injected at lateral tail vein with 1x 10^7 BM-MSCs cells in 0.2mL phosphate buffer saline (PBS) immediately following the induction of hypothyroidism[9].

3- Isolation and expansion of mice BM-MSCs:

The tibias and femurs of the mice were collected. Bone marrow samples were assembled from them. BM-MSCs were isolated and identified using flow cytometric and phenotyping analysis. BM-MSCs were then labeled using PKH-26 Red Fluorescent Cell Linker Kit (Sigma Aldrich, St.Louis, MO, USA).

4- Specimens collection:

Three weeks after stem cells injection, Rats of all groups were euthanized by an intra-peritoneal anesthetic overdose (sodium thiopental 80 mg/kg). Thus the total experimental period was 8 weeks; (5 weeks of hypothyroidism induction + 3 weeks of stem cells injection).

5- Specimens preparation:

The submandibular salivary glands from both sides were dissected and prepared for light microscopic examination. Specimens were fixed immediately in 10% formaldehyde solution for 24 hours, dehydrated, cleared and embedded in paraffin wax. Serial sections of about 5μm thickness were obtained and stained with hematoxylin and eosin [13].

RESULTS

Examination of histological sections of the submandibular salivary gland of control rats showed normal structure of its elements which were predominantly formed of seromucous acini. The acini appeared spherical in shape, deeply stained and coalesced to each other. Cells of acini appeared pyramidal in shape with basophilic and homogenous cytoplasm with rounded basally situated nuclei and clearly evident cellular boundaries. (Fig.1a)

The intercalated ducts were hardly distinguished because of their small size. They were lined by short cuboidal cells, with basophilic cytoplasm and large rounded centrally placed nuclei (Fig.1b). Meanwhile, striated ducts were clearly recognized between the acini, they were lined by single layer of columnar cells with intense acidophilic cytoplasm with basal striation and rounded centrally placed nuclei (Fig.1a).

The duct system of rat submandibular gland included granular tubules of unique character. They appeared larger than the striated ducts and exhibited irregular convoluted shape. The tubules were formed of columnar cells with basally situated nuclei and acidophilic cytoplasm. Abundant apical acidophilic granules were recognized giving the cells their granular appearance (Fig.1a).

The excretory ducts were located within the connective tissue septa between the lobules of the gland. They appeared large in diameter and lined by pseudo stratified columnar epithelium with scattered goblet cells, which were more distinctive in the large excretory ducts (Figs.1b).

On the other hand, histological Examination of H&E stained sections from submandibular salivary glands of group II revealed loss of normal glandular architecture with signs of deterioration and degeneration among the glandular elements. The acini were disorganized and widely separated, others were atrophied and shrunken. The nuclei appeared pyknotic and darkly stained or degenerated and some exhibited pleomorphism. Moreover, the cytoplasm showed decrease in its basophilia together with extensive vacuolation. Meanwhile, some acini revealed disruption of their cellular and acinar boundaries. Extravasated RBCs were clearly detected between the acini (Fig.2a).

The intercalated ducts assumed almost normal architecture (Fig.2a). On the other hand, the striated ducts appeared degenerated and collapsed with distorted ductal outlines revealing abnormal configuration (Fig.2b).Furthermore, the GCTs were markedly shrunken and degenerated with ill-defined cellular and ductal boundaries. Clumping of the glandular content together with extensive vacuolation and disorganized nuclei seemed to be persistent features (Fig.2b).

The excretory ducts showed disfigured and irregular outline. The epithelial lining showed loss of pseudostratification with disfigurement and flattening of some nuclei. Areas of cytoplasmic vacuolation were clearly visible. Furthermore, dilated blood vessels engorged with RBCs could also be detected within the dense fibrous connective tissue septa with inflammatory cells infiltration (Fig.2c).

After stem cells injection, the histological structure of the submandibular salivary glands showed close resemblance to that of the control group. To certain extent, the acini regained their former outline with reduction of spacing between them. Reappearance of definite cellular boundaries and normal nuclear configuration were distinguished within the acinar cells. Moreover, cytoplasmic vacuolation were hardly recognized compared to the previous group. However, very few degenerated and collapsed acini still exist within the parynchymal elements. Extravasated RBCs were also spotted intralobularly at close vicinity of the acini and ducts (Fig.3a).

Regarding the ductal system, the intercalated ducts preserved their normal appearance (Figs 3 a&b); On the other hand, the striated ducts showed remarkable improvement of their architecture with regain of basal striations. The nuclei returned their normal rounded shape. Nevertheless, few still exhibited flattened appearance. Few collapsed striated ducts were still evident in between the acini (Figs.2a&b). The GCTs restored their outlines with better arrangement of nuclei and lesser detected vacuolation (Figs.2b).

Apparently, the excretory ducts in between the lobules seemed regular and intact. They restored their normal pseudostratified appearance with uniform arrangement of cells. The connective tissue stroma appeared normal with no inflammatory cell infiltration. Engorged blood vessels were detected interlobularly adjacent to the excretory ducts (Fig.2c).

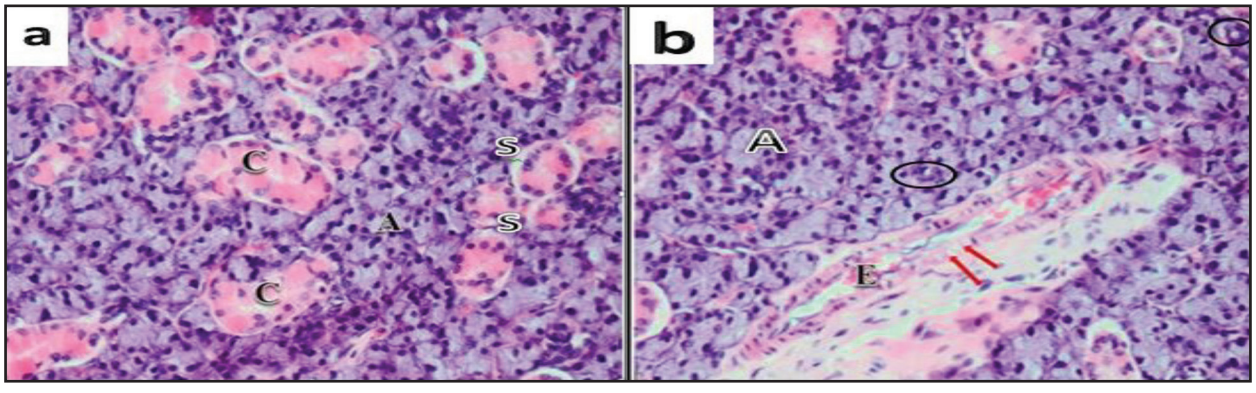

Figure (1): Photomicrograph of rat submandibular gland of group I showing: **a)** spherical deeply stained and coalesced serous acini (A), granular convoluted tubules with apical acidophilic granular material (C), striated duct with columnar cell lining and basal striations (S) .**b)** spherical deeply stained and coalesced serous acini (A), intercalated duct with short cuboidal cell lining (circle), excretory duct with normal pseudostratified lining (E), goblet cells (red arrows) (H&E, Orig. Mag.200).

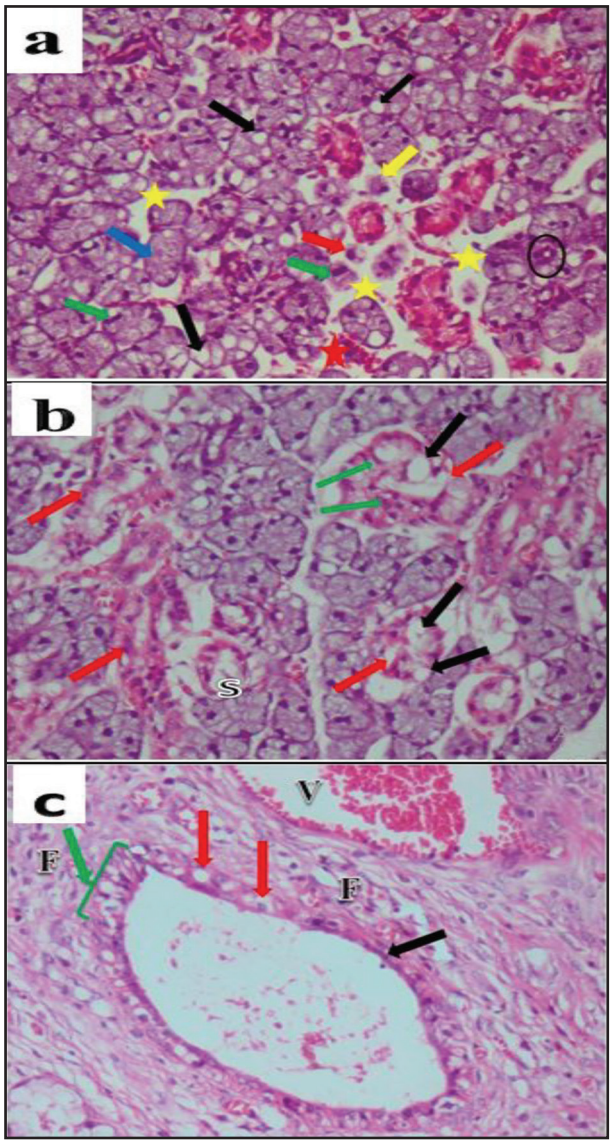

Figure (2) Photomicrograph of rat submandibular gland of group II showing: **a)**spacing between the acini (yellow stars), atrophied and shrunken acini (red arrow), disruption of acinar boundaries (yellow arrow), disruption of cellular boundaries (blue arrow), vacuolation through the acini (black arrows), darkly stained and pleomorphic nuclei (green arrows), Extravasated RBCs between the acini (red star), intercalated duct (circle). **b)** striated duct with distorted outline (S), destruction of the granular convoluted tubules with ill-defined cellular and ductal boundaries (red arrows), extensive vacuolation in their granular cytoplasm (black arrows), disorganized nuclei (green arrows). **C)** Disfigured lining of excretory duct (green arrow), flattened nuclei (black arrow) cytoplasmic vacuolation (red arrows), widely dilated and congested blood vessels (V), inflammatory cell infiltration (F) within dense fibrous connective tissue (H&E, Orig. Mag.200).

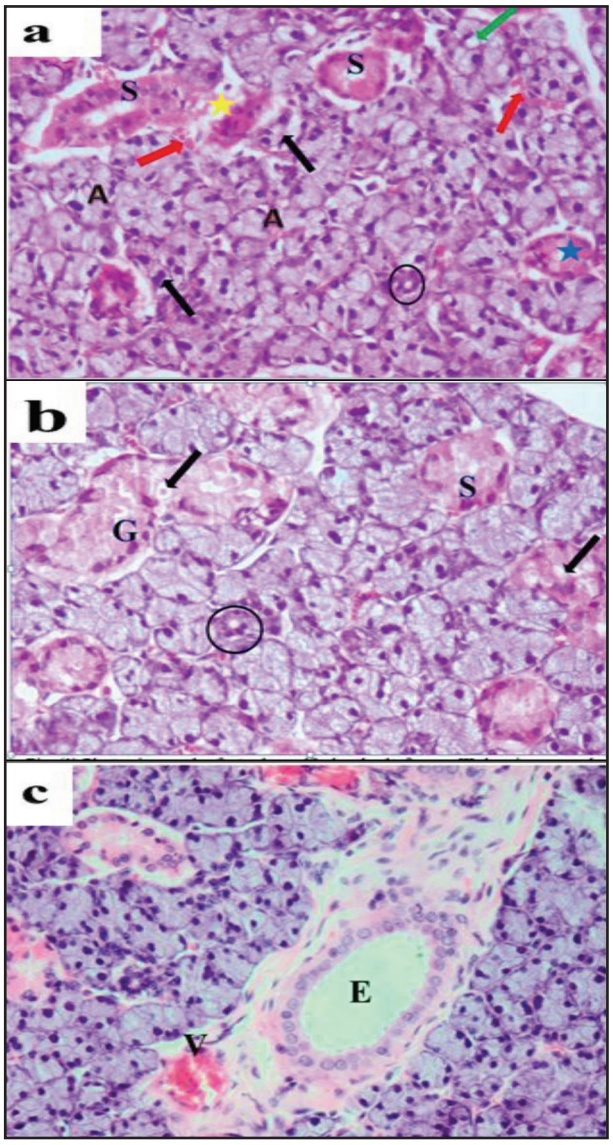

Figure (3) Photomicrograph of rat submandibular gland of group III showing: **a)** definite cellular boundaries and normal nuclear configuration of the acini (A), degenerated acini (black arrow), few areas of vacuolation (green arrow), extravasated RBCs (red arrows), uniform outline of the intercalated duct (circle), normal lining of striated duct (S), collapsed striated duct (yellow star), flattened nuclei of the striated duct (blue star). **b)** normal intercalated ducts (circle), normal striated ducts outline with some flattened nuclei (S), uniform outline of the granular convoluted tubules (G) with few vacuolation (black arrows). **C)** Normal excretory duct with pseudostratified columnar epithelium (E), engorged blood vessel (V) (H&E, Orig. Mag.200).

DISCUSSION

Hypothyroidism causes changes in the morphology and secretory function of the rat submandibular salivary gland. Authors have suggested that thyroid hormones are important in the maintenance of normal salivary gland function and histology [14].

Histological examination of group II (hypothyroid induced group) revealed atrophied, shrunken and widely separated acini and ducts. Consistent with these findings, decreased number and size of the acini has been noted in the salivary glands of hypothyroid rats induced by propylthiouracil and methimazole respectively [1, 2, 15].

Moreover, extensive vacuolation was clearly evident within the acinar and ductal cytoplasm of group II. This comes in agreement with previous study which reported shrinkage in the acini and cytoplasmic vacuolation in pancreatic tissue of hypothyroid rats [16].

Besides, in the present study the nuclei of the hypothyroid group appeared pyknotic and darkly stained or degenerated, and some exhibited pleomorphism. This foregoing observation goes in accordance with prior research that recognized degenerated cells with darkly stained cytoplasm and pyknotic nuclei in the parotid gland of hypothyroid rat [1].

All of these documented degenerative changes of the hypothyroid group may be related to the free radical damaging effect resulting from hypothyroidism [17].Certain investigation discovered that hypothyroidism caused an increase in plasma Malondialdehyde (MDA) formation (a product of lipid peroxidation) and a decrease in plasma superoxide dismutase (SOD) activity (an antioxidant enzyme) [18]. Increased MDA formation and decreased SOD activity suggested development of oxidative stress in hypothyroidism resulting in cell damage. In line with recent results that reported that the increase in lipid peroxidation products such as MDA led to cell death in renal tissue [19].

Differently, the histological structure of the submandibular salivary glands treated with BM-MSCs showed close resemblance to that of the control group. Marked improvement of the parenchymal elements after BM-MSCs injection was detected suggesting that BM-MSCs may ameliorate salivary glands damage following induction of hypothyroidism. Similarly, previous results reported that MSCs can preserve the acino-ductal integrity with secretory function following induction of hypothyroidism[20]. Additionally, recent study showed that intravenous transplantation of BM-MSCs rescue the functional damage of irradiated salivary glands [13].

Favorable results have been proved the capability of BM-MSCs to inhibit apoptosis, plus their potential to transdifferentiate into salivary epithelial cells [20]. Furthermore, another study added that BM-MSCs home to the damaged salivary glands and induced repairing processes by paracrine stimulation to improve glandular function and morphology [21].

CONCLUSIONS

From the previously mentioned results, the following could be concluded:

1. Hypothyroidism caused destructive changes of the submandibular salivary glands' parenchyma.
2. Systemic injection of BM-MSCs successfully regenerated hypothyroid-induced submandibular salivary gland acinar and ductal damage and restored the glands' architecture.
3. Systemic injection of BM-MSCs is considered as a promising treatment in regenerative medicine, regeneration in maxillofacial region and cell therapy protocols of hypothyroid dysfunction.

RECOMMENDATIONS

1. Further investigations on the potential effect and regenerative ability of BM-MSCs on longer durations.
2. Further investigations comparing the regenerative effect of other types of stem cells with

BM-MSCs on the degenerative changes of hypothyroid salivary glands.

3. Further studies on the synergetic effect of combined BM-MSCs treatment with natural herbal treatment on hypothyroid salivary glands.

No conflict of interest

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