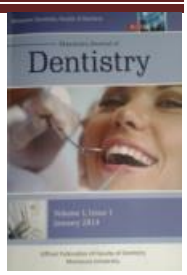
Effect of Bone Marrow Derived Mesenchymal Stem Cells on Albino Rats' Submandibular Salivary Gland After Intraglandular Injection of Bleomycin



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Abstract:
Objective: To evaluate effect of bone marrow mesenchymal stem cells (BMMSCS) on albino rats' submandibular salivary gland after intraglandular injection of bleomycin (BLM).
Methods: Forty- two rats were randomly divided into three groups. Group I (n=18): was subjected to intraglandular injection of a single dose of 1.5mg/kg of bleomycin. Group II (n=18): was subjected to intraglandular injection of a single dose of 1.5mg/kg of bleomycin dissolved in distilled water. After four weeks; rats were subjected to another injection of (1 x 10^5) cells of BMMSCs. Group III (n=6): was subjected to intraglandular injection of 0.05 ml of distilled water in the left submandibular salivary gland and rats were sacrificed after five weeks from starting the study. Tissue sections were stained with Toluidine blue then were subjected to digital image analysis followed by one- way ANOVA statistical analysis.

Introduction

Saliva is secreted by three paired sets of major glands and numerous of minor salivary glands. Saliva participate in digestion of carbohydrates and lipids, colonization of oral surfaces by bacteria and remineralization of teeth. Degeneration of ductal and acinar cells of salivary gland leads to salivary gland dysfunction [(1)].

Human salivary glands secretion is controlled by sympathetic, parasympathetic and hormonal stimulation [(2)]. Salivary glands are made of three epithelial cells types: acinar, ductal and myoepithelial. The acinar cells are responsible for fluid secretion, draining into the lumen of the ductal cells [(3)].

Saliva importance is obviously appeared in patients suffering from impaired function of salivary glands as a result of diseases such as diabetes, Sjögren's syndrome, medication side-effects and this leads to oral pain, dysphagia, reduced taste sensation and difficulty in speech and swallowing [(4)].

Actinobacterium Streptomyces verticillus bacteria produce bleomycin (BLM) which is anti-tumor and also is considered as a sclerosing agent. Pharmacokinetics of BLM is by oxidation in deoxyribose of thymidylate, which results in single-strand and double-strand breaks in DNA, chromosomal aberration, gaps, fragments and translocation [(5)].

Intralesional bleomycin injections have been shown to be an effective sclerosing agent for lymphatic malformations [(6)], vascular malformation, haemangiomas [(7)] and benign lymphoepithelial cysts of the parotid gland in HIV-positive patients [(8)].

Bleomycin may be injected in salivary gland accidentally or intentionally when the glands are involved within the lesions. It is observed that intraglandular injection of bleomycin leads to apoptosis, inflammation, fibrosis, edema, lipomatosis and congestion in the ductal and acinar cells of submandibular salivary gland [(9)].

Muscarinic agonist medications are traditionally used for stimulation of saliva secretion from the remnant functional parts of the gland. But they neither had an effect on the recovery of damaged tissues nor provided permanent relief of symptoms [(10)].

Stem cells are immature cells which have the ability to differentiate into multiple cell types [(11)]. Stem cells offer the possibility of a renewable source of replacement cells and tissues to treat a massive number of diseases and disabilities [(12)]. Direct transplantation of bone marrow mesenchymal stem cells (BMMSCs) into salivary glands can improve cytotoxic effect of chemotherapeutic agent on the salivary glands tissue [(13)]

Aim of the study:

This study was conducted to evaluate the effect of bone marrow derived mesenchymal stem cells on rats' submandibular salivary gland after intraglandular injection of bleomycin

Material and Methods

Animal subject:

Forty-two male white albino rats pathogen free (٢٠٠-250 g weight) were chosen. All steps were done under the protocol of ethical committee of Faculty of Dentistry, University of Mansoura, Egypt. Animals were received water and food.

Experimental protocol:

Group I: (n=18)

Rats were subjected to intraglandular injection of a single dose of 1.5mg/kg[124] of bleomycin dissolved in distilled water in the left submandibular salivary gland; then after four weeks six rats were sacrificed at 3 different times at 3, 7, 14 days.

Group II: (n=18)

Rats of this group were subjected to intraglandular injection of a single dose of 1.5mg/kg [124] of bleomycin dissolved in distilled water. After four weeks; rats were subjected to another injection of (1 x 10^5) cells of BMMSCs [125]. six rats were sacrificed at 3, 7, 14 days from BMMSCs injection.

Group III: (n=6)

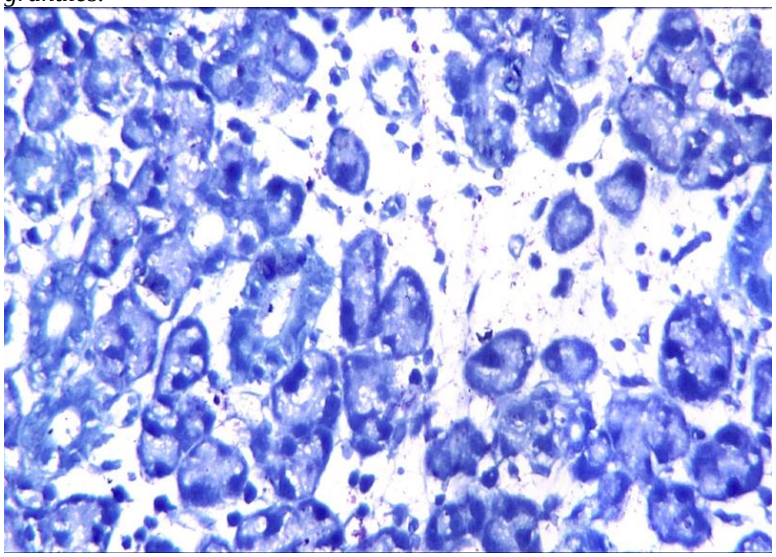
Rats of this group were subjected to intraglandular injection of 0.05 ml of distilled water in the left submandibular salivary gland. (sham group) They were sacrificed after four weeks.

Results:

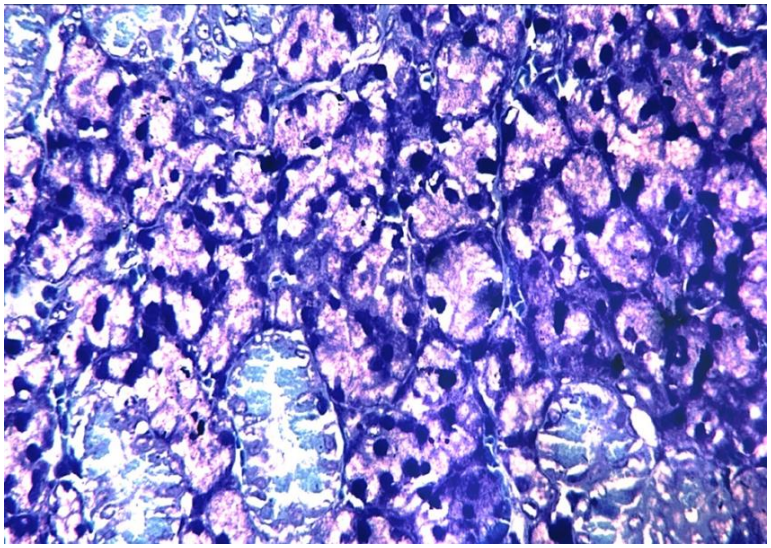
Group I: revealed ductal and acinar degeneration with loss of adhesion and loss of metachromatic secretory granules.

Group II: revealed more or less restoring ordinary architecture with maintenance distribution and adhesion of acini and large amount of metachromatic secretory granules.

Group III: ordinary architecture with maintenance distribution and adhesion of acini and large amount of metachromatic secretory granules.



Photomicrograph of group I showing loss of metachromatic secretory granules



Photomicrograph of group II showing very large amount of metachromatic secretory granules

One- way ANOVA analysis revealed significant difference between groups.

Table(1):Comparison of +ve cells between different studied groups.

	Group I		Group II		Group III		ANOVA P value
	%+ve		%+ve		%+ve		
	Mean	±SD	Mean	±SD	Mean	±SD	
+ve cell%	2.593	0.599	20.762	5.794	0.709	0.118	<0.001*
P1			<0.001*		<0.001*		
P2					0.001*		

Data expressed as mean±SD SD: standard deviation P: Probability
**: significance <0.05 (Test used: One- way ANOVA followed by post-hoc Tukey)*
P1: significance relative to Group I P2: significance relative to Group II

Discussion:

Toluidine blue staining was used to reveal the density of secretory granules of serious acini of submandibular salivary gland **(Jeong et al., 2017).**[14] Group I result of toluidine blue showed that low density of secretory granules at the four different time intervals and this might be due to the degenerative effect of bleomycin on salivary gland. **(Vissink et al., 1991)** improved that exposure of submandibular gland to acute irradiation resulted in morphological and functional changes. [15]

Another agreement with our group I results were by **(EL-Kordy et al., 2014)** who demonstrated that marked atrophy of the acinar components with loss of their metachromatic material of submandibular gland after 7 days following starvation. [16]

Wang et al., 2018 they suggested their findings according to the ability of stem cells to stimulate tissue repair via paracrine activities and vasculogenesis. Also, it has the ability to differentiate into endothelial cells, secrete angiogenic growth factors, such as hepatocyte growth factor and vascular endothelial growth factor.[17]

Conclusion: These results suggest that BMMSCs may represent a novel and effective therapy for salivary gland degeneration and fibrosis caused by bleomycin that needs to be further investigated in humans.

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