The Effect of Transplanted Bone Marrow Stem Cells on the Tongue of Irradiated Rats (Histological and Immunohistochemical study)

Aboushady, I.M.[1]; Mubarak, R.T. [1,2]; El-mougy, S. A. F. [1]; Rashed, L.A.[3]; El-desouky, A.A.[4]

[1]Oral Biology Department, Faculty of Oral & Dental Medicine, Cairo University.
[2]Oral Biology Department, Faculty of Oral & Dental Medicine, Nahda University.
[3]Biochemistry Department, Faculty of Medicine, Cairo University.
[4]Surgery, Anesthesia and X-Ray Department, Faculty of Veterinary Medicine, Cairo University.
eimoshady@hotmail.com

Abstract: Normal tissue damage following radiotherapy (RT) is still a major problem in cancer treatment. So, the current work aimed at exploring the possible role of locally injected bone marrow derived mesenchymal stem cells (BM-MSCs) in ameliorating the side effects of ionizing radiation on the normal rat's tongue. **Materials & Methods:** Ten rats were used for isolating BM-MSCs, ten rats served as control group (G1) and twenty rats received a single radiation dose of 10 Gy to the head and neck region, then, they were equally divided into 2 experimental groups: Irradiated only group (G2) and Irradiated+MSCs group (G3). All animals were sacrificed at 2 weeks following irradiation. The tongue was examined histologically and immunohistochemically using anti-PCNA primary antibody. **Results:** Histological & immunohistochemical examination of the treated group (G3) revealed an obvious improvement in the histological structure of the tongue; compared to the irradiated group (G2); in addition to up regulated expression of PCNA, indicating improved cell proliferation rate. **Conclusions:** BM-MSCs have shown positive effect in protection against the side effects of radiotherapy on normal tissues, which was emphasized by their enhancing effect on the proliferative capacity of these tissues.

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Key Words: Tongue; ionizing radiation; BM-MSCs; PCNA.

1. Introduction

Stem cell therapy is emerging as a potentially revolutionary new way to treat disease and injury, with wide-ranging medical benefits. It aims to repair damaged and diseased body parts with healthy new cells provided by stem cell transplants. By definition, a stem cell is a cell that has the ability to divide (self replicate) for indefinite periods, often throughout the life of the organism. Under the right conditions, or given the right signals, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells (**Slack, 2000)**.

Apart from the bone marrow (BM), MSCs are located in other tissues like: adipose tissue, peripheral blood, cord blood, liver and fetal tissues **(Bobis *et al.*, 2006)**. Given the recent reports of the plasticity of BM-MSCs, their therapeutic potential has obviously been brought into the spotlights of many research fields. One possible application of stem cell research is the repair of tissue injuries related to the side effects of radiotherapy and hence, ameliorating radiation-induced complications.

Radiation therapy to the head and neck frequently results in serious and sometimes unavoidable changes to the orofacial structures. Based on the usual time of their occurrence, radiation-induced changes can be divided into: early or acute side effects which are noted during or shortly after treatment, affecting mucosa, taste and salivary glands; and late side effects which develop months or years after the end of radiation therapy, affecting salivary glands, teeth, bone, muscles and skin **(Dörr *et al.*, 2002)**.

So, the present work was designed to provide a new research platform through exploring the histological effect of BM-derived MSCs transplantation on the structure of the tongue of irradiated rats. Also, this study investigated the effect of this therapy on the proliferative capacity through the expression of anti-PCNA antibody in the rat's tongue under the same experimental conditions, using immunohistochemical technique.

2. Materials &Methods

Forty male rats weighing about 100-150 grams were used in this study. The animals were bred at the Research Institute of Ophthalmology. They were housed in stainless steel cages and were maintained under good ventilation. All rats had been fed on standardized laboratory balanced diet and were given water *ad libitum.*

Experimental Design:

Ten rats were used for isolation of BM-MSCs, ten rats served as control group (G1), the remaining twenty rats received a single radiation dose of 10 Gy to the head and neck region, then they were equally divided into two groups: Irradiated group (G2) and Irradiated+MSCs group (G3), the later received immediate, single, local injection of 0.25 ml BM-MSCs (in PBS) at the ventral surface of the tongue.

Rat Anesthesia:

The rats were anaesthetized by i.p. injection of sodium pentobarbital (Nembutal®, 40 mg/kg body weight) and ketamine chloride (ketalar®, 40 mg/kg body weight) **(Ikeguchi *et al.*, 2006)**.

Radiation Exposure:

Irradiation of the rats was performed at the National Cancer Institute (NCI), Cairo, Egypt, using a Cobalt 60 source [energy 1.25 MV, THERATRON 780 E] giving a dose rate of about 139cGy/min at the time of experiment.

Preparation of BM- MSCs:

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male rats with DMEM (GIBCO/BRL) supplemented with 10% fetal bovine medium **(**GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and were resuspended in a complete culture medium supplemented with 1**%** penicillin-streptomycin **(**GIBCO/BRL**)**. The cells were then incubated in a CO_2 incubator at 37□ C in 5% humidified CO_2 for 12- 14 days 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) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 minutes at 37□ C. After centrifugation, cells will be resuspended in PBS. MSCs in culture are characterized by their adhesiveness, fusiform shape and by detection of CD29, one of the surface markers for MSCs by RT-PCR **(Rochefort *et al.*, 2005)**.

Labeling Of Stem Cells with PKH26 Dye:

MSCs cells were harvested during the 4th passage and were labeledwith PKH26 fluorescent linker dye. PKH26 is a red fluorochrome having 551 nm excitation and 567 nm emission. The labeled cells retain both their biological and proliferating activities. Thus, the linker is ideal for *in vitro* cell labeling, *in vitro* proliferation studies and long term, *in vivo* cell tracking. The dye itself is stable and will divide equally when the cells divide.

Detection of homing of the injected cells in the rat's tongue:

At the endpoint of the study, the rat's tongue was examined with a fluorescent microscope to detect the cells stained with PKH26 dye to ensure engraftment of the injected cells into the irradiated tongue **(Fig. 1)**.

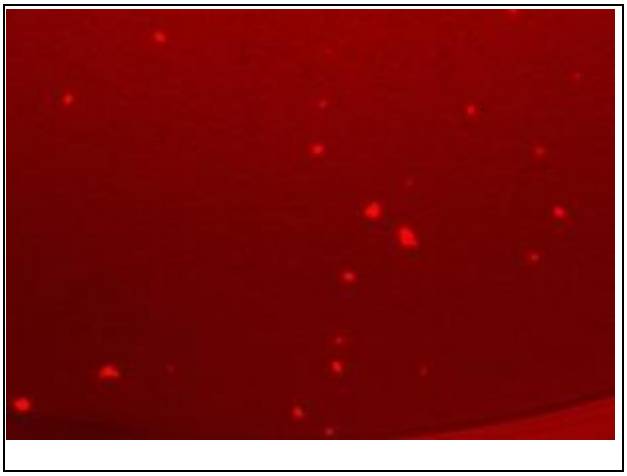


Fig. (1): Cells labeled with PKH 26 showing strong red auto fluorescence at 2 weeks following transplantation in the rat's tongue.

Specimen preparation:

All animals were sacrificed by cervical dislocation at two weeks following irradiation; the tongue was dissected out and fixed in 10% neutral buffered formalin. Specimens were then dehydrated through graded alcohol, cleared in xylene and embedded in paraffin. Sections of 4-6µ thickness were prepared and subjected to haematoxylin and eosin staining for routine histological examination.

Immunohistochemistry:

Sections of 4µ thickness were mounted over electrically charged slides, then fixed in a 65 ^{o}C oven for 1 hr. Slides were immersed in 60ml of Triology working solution (Cell Marque, CA-USA. CAT. # 920p-06); a product that combines the three pretreatment steps: deparaffinization, rehydration and unmasking; at 120 ^{o}C for 15 min after which the slides were allowed to cool for 30 min. Sections were then washed and immersed in TBS to adjust the pH, this is repeated between each step of the IHC procedure. Quenching endogenous peroxidase activity was performed by immersing the slides in 3% hydrogen peroxide for 10 min. Broad spectrum LAB-SA detection system from Invitrogen (Cat # 85-9043) was used to visualize any antigen-antibody reaction in the tissues. Background staining was blocked using 10% goat non immune serum blocker for 10 min. Sections were then incubated with primary antibody: anti-PCNA (Ab-1 (Clone PC10), CAT. # MS-106-R7) obtained from lab vision (Lab Vision Corporation 46360 Fremont Blvd. Fremont, CA 94538-6406, USA).

Henceforward, biotinylated secondary antibody was applied for 20 min followed by 20 min

incubation with the enzyme conjugate. DAB chromogen was then applied for 2 min, rinsed, after which counterstaining and cover slipping were performed. All slides were examined with light microscopy for histological evaluation and for detection of the specific brown reaction product of the DAB substrate in the immunostained sections.

Immunostaining Interpretation by Image Analysis:

The immunostained sections were examined using an image analyzer computer system to assess the optical density of the immunostain. The image analysis was performed using a computer (software Leica Quin 500) The image analyzer was first calibrated automatically to convert the measurement units from pixels into actual micrometer units.

The intensity of the reaction within the cells was determined by measuring the optical density in 10 small measuring fields in each specimen using a magnification of 400. And finally, mean values were obtained for each case.

Statistical Analysis:

All the obtained data from the computer image analysis were statistically evaluated. These data represented the value of PCNA immunoexpression and immunostaining intensity. They were given as mean values ± SD (standard deviation).

The analysis of variance (ANOVA) test was used to compare the mean immunostaining intensity values of PCNA between the control and the experimental groups. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results

Histological Results:

Light microscopic examination of the rat's tongue of the control group (G1) revealed the oral mucosa covering the underside of the rat's tongue being composed of keratinized stratified squamous epithelium, having a thin layer of keratin and short epithelial ridges. Besides, the lamina propria was generally thin, contained few collagen fibers and small blood vessels. Groups of well formed striated muscles were also noticed beneath the lamina propria **(Fig. 2)**.

In the Irradiated group (G2), the ventral surface of the tongue revealed an obvious reduction in the thickness of the keratin and the epithelium, the supporting basement membrane was almost straight and congested blood vessels were detected in the underlying lamina propria **(Fig. 3)**. Even, a focal ulceration occasionally occurred within the epithelium, which was accompanied by massive chronic inflammatory cell infiltration and an apparent increase in the keratin thickness opposite to the ulcerated area. **(Fig. 4)**. Besides, the lingual muscles showed obvious atrophy in many areas, together with increased collagen fiber deposition in between **(Fig. 5)**.

In the Irradiated+MSCs group (G3), the ventral surface of the tongue showed an apparent increase in the thickness of both the epithelium and the covering keratin than that in the irradiated group; but was still reduced than that of the control group. Also, the C.T. papillae appeared well defined and the lamina propria revealed fewer dilated blood vessels **(Fig. 6)** compared to the case in the irradiated group. Besides, the lingual muscles appeared to be well formed in most areas of the tongue and less marked fibrous deposition in between the bundles occurred; in contrary to those of the irradiated group **(Fig. 7)**.

Immunohistochemical Results:

In the control group (G1), the ventral surface of the tongue displayed strong positive nuclear PCNA immunostaining among the basal cells while few cells in the lower part of the prickle cell layer presented mild to moderate nuclear immunostaining. In the lamina propria, few cells revealed strongly stained nuclei **(Fig. 8)**.

In the irradiated group (G2), the ventral surface revealed an obvious decrease in the number of nuclei showing immunoreactivity among the basal cells where the immunoreactivity to PCNA was mild to moderate, while in the lamina propria, PCNA +ve nuclei were almost absent **(Fig. 9).**

In the irradiated+MSCs treated group (G3), most basal and suprabasal cells showed strong PCNA nuclear immunoreactivity. Few –ve nuclei could be detected and some cells in the lower part of the prickle cell layer displayed mild to moderate reaction while in the lamina propria, weak to mild cytoplasmic reactivity was observed in few cells **(Fig. 10)**.

Statistical Results (Table I and Graph I):

Two weeks following irradiation of the rats, the mean value of PCNA (mean ± SD = 54.24 ± 11.71) showed significant decrease than that of the control group (mean ± SD = 77.16 ± 0.41) as the *P*-value was < 0.01.

While the mean value of PCNA, in the treated group (mean ± SD = 76.13 ± 4.38), was significantly increased than that of the irradiated group (mean ± SD = 54.24 ± 11.71) and was not significantly decreased than that of the control group (mean ± SD = 77.16 ± 0.41) as the *P*-value was >0.05.

4. Discussion

The goal of radiation treatment is to deliver completely measured doses of irradiation to a defined tumor volume, with the minimum accepted injurious effects of irradiation to the surrounding healthy tissues, through eliminating the tumor cells, thus giving a high quality to life and prolonging survival at reasonable cost to cancer patients **(Ertekin *et al.*, 2003)**.

Radiotherapy (RT) plays an important role in the treatment of patients with head and neck cancer. Depending on the location of malignancy, unavoidably, the salivary glands, the oral mucosa and the jaws have to be included in the radiation portals. As a result, changes induced by exposure to radiation occur in these tissues. The resulting oral sequelae include hyposalivation, mucositis, loss of taste, osteoradionecrosis, radiation caries and trismus. These sequelae may be dose limiting and may have tremendous effect on the patient's quality of life **(Vissink *et al.*, 2003)**.

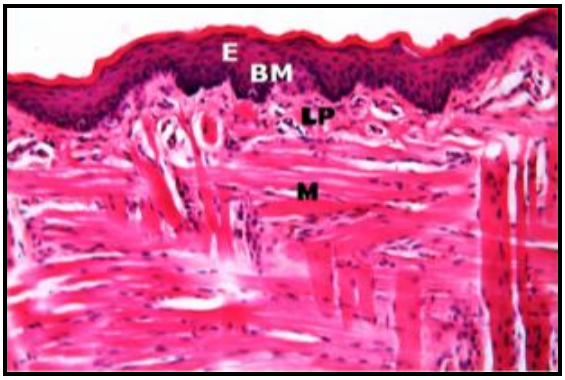


Fig. (2): Photomicrograph of the tongue of the control group (G1) showing: keratinized stratified squamous epithelium covering the ventral surface (E), lamina propria (LP), basement membrane (BM) and well formed striated muscle bundles (M) (H&E, Orig. Mag. 200).

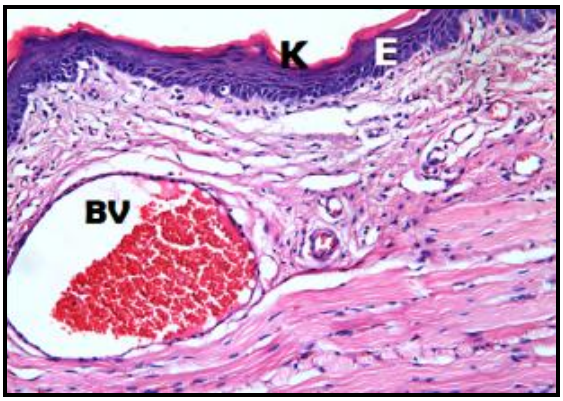


Fig. (3): Photomicrograph of the tongue of the irradiated group (G2) showing: apparent reduction in the thickness of both the keratin (K) and the epithelium (E) covering the ventral surface of the tongue and congested blood vessel (BV) (H&E, Orig. Mag. 200)

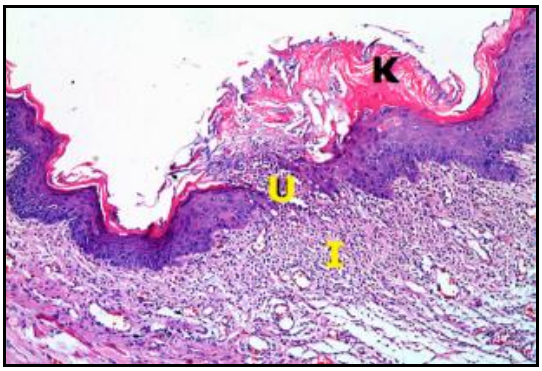


Fig. (4): Photomicrograph of the tongue of the irradiated group (G2) showing: ulcer formation (U), together with massive chronic inflammatory cell infiltration in the underlying lamina propria (I) and increased keratin thickness opposing the ulcer (K) (H&E, Orig. Mag. 100)

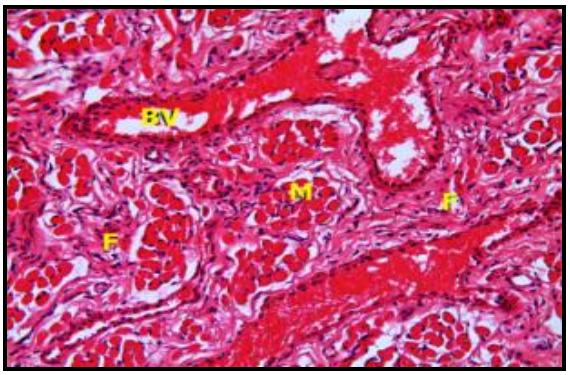


Fig. (5): Photomicrograph of the tongue of the irradiated group (G2) showing: obvious atrophy among the lingual muscles (M) with increased collagen fibers deposition in between (F), together with markedly congested blood vessels (BV) (H&E, Orig. Mag. 200)

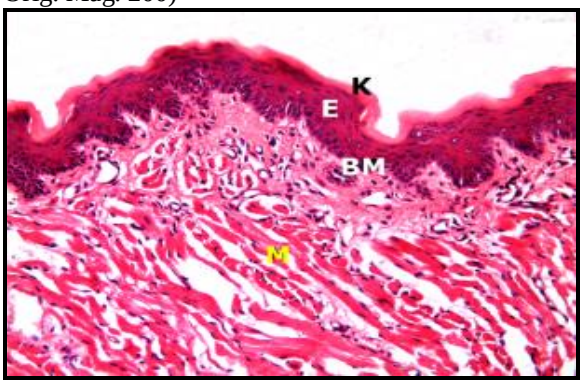


Fig. (6): Photomicrograph of the tongue of the treated group (G3) showing: an apparently increased thickness of both the keratin (K) and the epithelium (E) covering the ventral surface, well defined regular basement membrane (BM) and well formed muscle bundles (M) (H&E, Orig. Mag. 200)

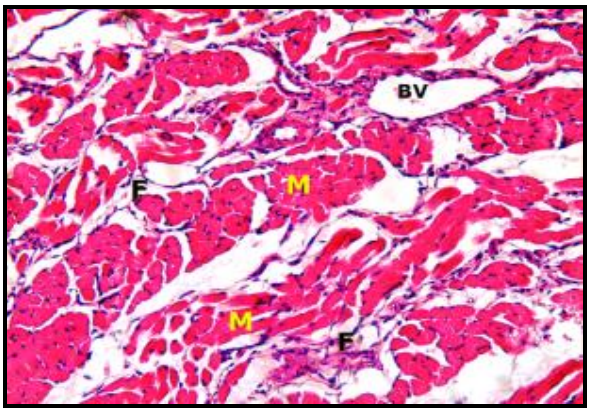


Fig. (7): Photomicrograph of the tongue of the treated group (G3) showing: dilated blood vessel (BV), well developed muscles (M) with less fibrous deposition in between (F) (H&E, Orig. Mag. 200)

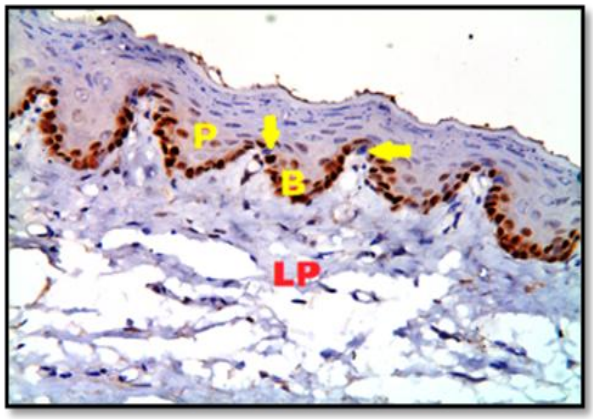


Fig. (8): Photomicrograph of PCNA immunolocalization in the tongue of the control group (G1) showing: strongly positive nuclei in the basal cells (B) and few prickle cells (P), few –ve nuclei (yellow arrows) and mild reaction in the lamina propria (LP) (DAB, Orig. Mag. 400).

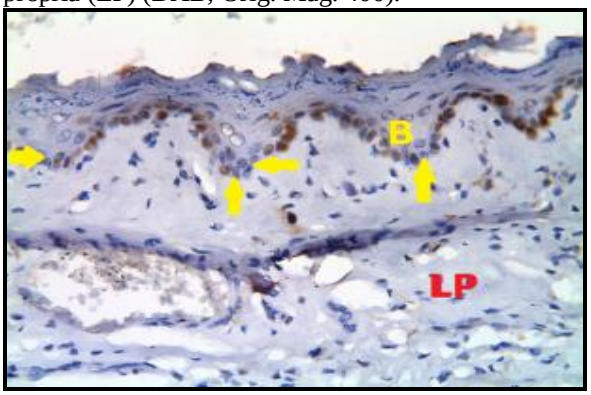


Fig. (9): Photomicrograph of PCNA immunolocalization in the tongue of the irradiated group (G2) showing: more -ve nuclei (yellow arrows) among the basal cells (B) and the lamina propria (LP) displayed weak cytoplasmic immuno-reactivity (DAB, Orig. Mag. 400).

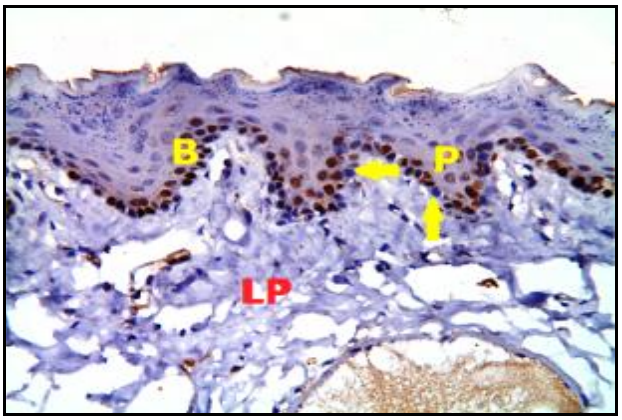
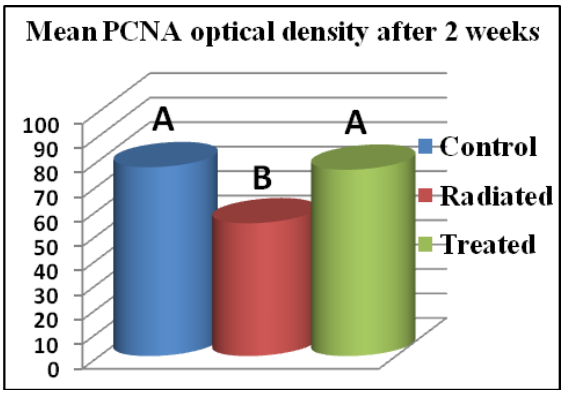


Fig. (10): Photomicrograph of PCNA immunolocalization in the tongue of the treated group (G3) showing: few –ve nuclei (yellow arrows) among the basal cells (B), few prickle cells (P) displayed positivity, while the lamina propria (LP) revealed weak to mild cytoplasmic reaction (DAB, Orig. Mag. 400).

Table I: The difference in mean PCNA optical density between Control, Radiated and Treated groups after 2 weeks using ANOVA Test.

Group	PCNA optical density		
	M±SD	F-Value	*p*-Value
I-Control	77.164±0.414	13.31	0.0009
II-Radiated (2weeks)	54.236±11.706		
III-Treated (2weeks)	76.126±4.385		

**Significant difference at (p<0.001).
M1 vs M2 P<0.01
M1 vs M3 non significant
M2 vs M3 P<0.01



Graph I: Represents the difference in mean PCNA optical density between different groups after 2 weeks. Groups sharing a letter aren't significantly different from each other.

Thus, there is an absolute necessity to investigate a novel method, with both efficacy and safety, for preventing &/or treating the side effects occurring during cancer treatment, which, in turn, throws light on the possibility of using stem cell therapy in this field.

Researchers have looked for years for ways to use stem cells to replace cells and tissues which are damaged or diseased. Hence, stem cells have recently received much attention **(Kumar & Singh, 2006)**. Stem cells have the remarkable ability to self-renew, as well as to differentiate into multiple cell types in response to extracellular signals. Thus, they hold a great promise in regenerative medicine **(Jaishankar & Vrana, 2009)**.

So, the present work was designed to explore the histological effect of BM-MSCs transplantation on the structure of the tongue of irradiated rats. Also, the effect of this therapy on the expression of PCNA antibody was addressed in the current investigation.

In the oral cavity, areas with large blood supply or high cell turnover rate and hence, respond more intensely to radiation include: the lateral borders and the ventral surface of the tongue as well as the soft palate and the floor of the mouth. **(National Cancer Institute, 2002)**. Besides, animal models for radiation-induced oral mucositis frequently used mice, which are techniquely difficult to manipulate due to the small size of the typical mouse **(Sciubba & Goldenberg, 2006 and Zhao *et al.*, 2009)**. Thus, based on the preceding observations, the rat's tongue was the tissue of choice in the present work as it provides the best reproducibility and adequate space for sufficient tissue sampling.

The first challenge in MSCs transplantation is that the cultured cells retain their quality and their differentiation potential during the expansion process. Under optimal conditions, MSCs can be maintained in culture for 20-30 population doublings and still retain their capacity for differentiation **(Friedenstein *et al.*, 1970)**.

MSCs are ideal for stem cell-based therapy because of their off-the-shelf availability. Apparently, these cells might be simply isolated from various tissues and expanded in culture in large numbers **(Wakitani *et al.*, 1994 and Young *et al.*, 1998)**. These cells also represent an appropriate route to avoid immunorejection since they are immunopriviledged i.e. escape detection from the immune system **(Le Blanc *et al.*, 2003)**. These cells are also less probable to trigger teratoma formation and they are more ethically accepted compared to the use of embryonic stem cells **(Bobis *et al.*, 2006)**.

Bone marrow derived-MSCs (BM-MSCs) are one of the first known MSCs and are also the most advanced in clinical trials. For these reasons, these cells generally serve as the 'gold standard' against which, other MSCs sources are compared **(Klingemann *et al.*, 2008)**.

In the present work, local route was chosen to deliver the BM-MSCs directly into the rat's tongue to be sure of the successful engraftment of the injected cells in the tongue, which was further emphasized by the demonstration of the PKH26 dye in the tongue.

Several studies supported the utility of local MSCs injection in the improvement of many conditions. For example, **Azizi *et al.*, (1998)** reported that following direct injection of MSCs into the rodent's brain, the cells differentiated into glial populations. In addition, local administration of MSCs into the heart generated de novo myocardial formation, thus giving the hope of using these cells in treating coronary heart diseases **(Orlic *et al.*, 2001)**.

Immediate injection of MSCs was preferred in the present investigation to ensure efficient homing of the injected cells into the tongue, based on the previous report by **Konoplyannikov *et al.*, (2008)** who demonstrated that irradiation stimulated homing of the injected cells more than in the non-irradiated rats. They also reported that the intensity of homing decreased with prolongation of the period between irradiation and systemic transplantation.

In the present work, light microscopic examination of H & E stained sections of the irradiated tongue revealed an apparent decrease in the thickness of the epithelium and the covering keratin. In addition, the basement membrane was less defined and almost straight in some areas. Occasionally, an ulcerated area occurred which was accompanied by massive chronic inflammatory cell infiltration in the underlying lamina propria as well as increased keratin deposition opposite the ulcer.

Congested blood vessels were also noticed in the lamina propria and between the lingual muscles. Besides, areas of muscle atrophy were detected among the lingual muscles associated with increased fibrous deposition.

The apparent reduction observed in the epithelial thickness of the irradiated rat's tongue in the present work agrees with the reduced mucosal basal layer cellularity and the reduced mucosal thickness in the ventral surface of the tongue of mice at 4 days following radiation, as reported by **(Zhao *et al.*, 2009)**. Moreover, **Li *et al.*, (2011)** developed a RT-induced oral mucositis rat model where at 7-15 days, infiltrating cells, necrosis and exfoliation of squamous cells occurred and an ulcerative lesion could be observed.

In the present study, immuno-histochemical examination of the irradiated rat's tongue revealed an obvious reduction in PCNA expression compared to

that in the control group and statistical results showed that this decrease was highly significant.

Proliferating cell nuclear antigen (PCNA) is a 36 KDa, 261-aminoacid non-histone polypeptide that is localized in the nucleus and is associated with cell proliferation, functioning as an auxillary protein to DNA polymerase-delta. PCNA appears to be necessary for DNA replication and elevated levels of this protein at the G_1/S phase transition are present in cells undergoing division **(Gelb *et al.*, 1992 and Siitonen *et al.*, 1993)**.

In agreement with the current results, in an *in vitro* model to study the effects of radiation on oral keratinocytes and hence, simulating RT-induced oral mucositis, **Tobita *et al.*, (2010)** demonstrated that irradiation decreased the proliferative capacity of the cultured cells, with few cells noted in mitosis, eventually leading to decreased epithelial thickness. These observations were based upon the direct correlation demonstrated between the decreased proliferative activity and the decreased number of Ki-67 immunopositive cells, which in turn decreased in proportion to increasing the radiation dose. In addition, **Oh *et al.*, (2010)** demonstrated decreased expression of PCNA at 5&7 days following radiation in a RT-induced intestinal injury mice model.

In contrary to these observations, **Lyng *et al.*, (2004)** reported enhanced PCNA expression in primary cultures of rainbow trout skin following irradiation. The authors added that the percentage of darkly stained PCNA +ve cells increased significantly as radiation dose increased. They concluded that increased PCNA expression with increasing radiation dose indicated enhanced proliferation after irradiation.

According to the present work, light microscopic examination of the MSCs treated rat's tongue (G3) revealed an obvious improvement in the histological picture compared to the irradiated group as shown by the apparent increase in the thickness of the epithelium and the covering keratin, as well as the presence of more defined basement membrane. And regarding the lamina propria, congested blood vessels and inflammatory infiltrate became less frequently observed and the lingual muscle bundles were well developed in most areas.

Immunohistochemical results of the MSCs treated group supported the obtained histological results, as the PCNA expression in this group was markedly increased than that in the irradiated one and this increase was statistically significant. Thus, in the current study, based upon the observed upregulation in PCNA expression of the treated group, the injected BM-MSCs seemed to promote proliferation of the epithelial cells.

In accordance with the obtained results, it was reported by **Herrera *et al.*, (2004)** that I.V. administered MSCs enhanced tubular proliferation in acute renal failure as detected by the increased number of PCNA +ve cells, with a significant contribution of the engrafted MSCs in the tubular epithelial cells regeneration. Moreover, **Tögel *et al.*, (2005)** demonstrated higher proliferative and lower apoptotic indices, lower renal injury and significantly improved renal function following intracarotid MSCs administration after renal ischemia.

In the present study, the obvious improvement observed in both, the histological and immunohistochemical features of the MSCs treated group (G3) could be explained according to various human and animal studies which dealt with the therapeutic potential of MSCs to repair normal tissue injuries related to the side effects of radiotherapy. For instance, **Bensidhoum *et al.*, (2005)** reported reduced damage and accelerated wound healing process in the skin of irradiated mice after their I.V. injection with human MSCs (hMSCs) 24 hours after irradiation. The authors also demonstrated the presence of the injected hMSCs in the scar as they participated in wound healing.

In addition, **Françoise *et al.*, (2006)** have suggested that radiation-induced tissue injury can be ameliorated when MSCs are infused, where the irradiated rats which were given MSCs had a faster recovery from RT-induced small intestinal and liver damage; and a faster wound healing. Moreover, **Bey *et al.*, (2010)** found that in a very severe radiation burn, combining skin autograft with 5 local MSCs administrations resulted in a favorable clinical outcome and no recurrence of radiation inflammation during the patient's 8-month follow up. The authors concluded that the benefit of this local cell therapy could be linked to the "drug cell" activity of MSCs by modulating the radiation inflammatory process.

In the same year, **Kudo *et al.*, (2010)** demonstrated that almost full recovery of radiation-induced intestinal injury occurred in irradiated mice which received MSCs injection into the intestinal wall immediately following irradiation. The authors suggested that the injected MSCs may transfer intestinal stem like cells to the radiation-induced intestinal injury and contribute to fibroblasts proliferation. Then, FGF and other anti-inflammatory cytokines could be induced which, in turn, can repair the injury.

Although the exact mechanism of stem cells action in regeneration can't be actively investigated in humans, animal models have provided a large number of explanations for the observed treatment effects. **Rabb, (2005), Chien, (2006) and Tögel *et al.*, (2007)** have proposed the potential mechanisms of BM-MSCs action, which included: the secretion of a number of cytokines, chemokines and growth factors which are

potentially disease modifying. Subsequent actions of these factors include stimulation of angiogenesis, suppression of inflammation, inhibition of apoptosis and enhancement of endogenous repair by stimulating intrinsic stem and progenitor cell proliferation.

Similarly, **Guo *et al.*, (2007)** confirmed the anti-inflammatory role of MSCs transplantation by demonstrating decreased gene expression of the inflammatory cytokines TNFα, IL-1β & IL-6. While **Lee, in (2008)** was the first to demonstrate the favorable, antioxidant, ROS-scavenging capacity of MSCs. However, the detailed mechanism of the protective role of MSCs in radiation-induced injury still remains unclear.

Conclusion

Ionizing irradiation, in addition to its anti-tumor effects, causes damage in normal tissues located in the radiation field. This becomes particularly evident in the head and neck region, a complex area composed of several dissimilar structures that respond differently to radiation. Although thorough protocols have been developed to manage the early and late oral sequelae of radiotherapy of the head and neck region, these sequelae are still difficult to manage.

Stem cells pose a bright future for the therapeutic world by providing a promising treatment for the diseases which are considered as non curable nowadays. However, unresolved issues such as lack of conformity of isolation and *in vitro* culture protocols, in addition to the heterogeneity of MSCs populations, continue to be obstacles in the field of MSC therapy.

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