



Evaluation of Local Versus Intravenous Administration of Adipose Tissue-Derived Stem Cells on Regeneration of Oral Epithelium of Chemotherapy Treated Rats

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ABSTRACT

Purpose: This study aimed to evaluate the effect of local and intravenous administration of adipose tissue-derived stem cells on the regeneration of oral epithelium in chemotherapy-treated rats. **Material and Methods:** 48 adult male albino rats were used in this study throughout the experimental period (2 weeks). 12 rats were left as negative controls (G I) and the remaining experimental 36 rats were treated with 5-fluorouracil (chemotherapeutic agent) in a dose of 50 mg/kg/dose to induce oral mucositis. Moreover, acetic acid was injected in the buccal mucosa to produce ulcers and then the rats were subdivided into three subgroups, (G II) in which 12 rats received no treatment, (G III) in which 12 rats received a direct injection of a single dose of adipose-derived stem cells, (G IV) in which 12 rats received an intravenous injection of a single of adipose-derived stem cells. All rats were euthanized; cheek epithelium was dissected and processed for hematoxylin & eosin evaluation. **Results:** Group III produced better healing than group IV where the ulcer appeared covered with a continuous layer of regenerated epithelium after one week in group III while the regenerating epithelium failed to cover the ulcer in group IV. After two weeks, the epithelium covered the ulcers in both groups but the C.T. architecture appeared better in group III. **Conclusion:** The direct injection of ADSCs in oral mucositis produces faster healing potentiality than intravenous injection.

KEYWORDS

Oral mucositis, Ulcer,
ADCs, 5-FU.

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INTRODUCTION

The oral epithelium serves as a buffer between the underlying tissues and their surroundings. The surface squamous epithelium and the deeper lamina propria are the two layers that make up the oral mucosa. The buccal mucosa of rats is covered by keratinized epithelium. The epithelium of the keratinized oral mucosa consists of four layers: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum ⁽¹⁾.

5-fluorouracil (5-FU) is a fluoropyrimidine antimetabolite drug that is used usually as cancer therapy, especially colorectal cancer. 5-FU inhibits thymidylate synthase (TS) and causes its metabolites to be incorporated into RNA and DNA, which has anti-cancer properties ⁽²⁾.

Mucositis is a devastating consequence of antineoplastic therapies caused by the epithelial mucosa's inflammatory response to the cytotoxic effects of chemoradiotherapy. Mucositis affects about 40% of patients who take chemotherapy, and it distresses about 90% of patients who received both chemo and radiotherapy ⁽³⁾.

As a consequence of their capability to self-renew and differentiate into tissue-specific cells including osteoblasts, chondrocytes, and adipocytes, mesenchymal stem cells (MSC) have numerous promises in regenerative medicine. MSCs were utilized in musculoskeletal regenerative therapy to treat age-related orthopedic degenerative illnesses and other conditions ⁽⁴⁾.

With the progression of stem cell technology, new therapeutic approaches for a diversity of diseases have lately become practicable. Human mesenchymal stem cells (MSC) and, in particular, adipose-derived mesenchymal stem cells (Ad-MSC) have gained countless interest among stem cell therapies because of their comparatively no adverse reactions ⁽⁵⁾.

Though the advance of the MSC sector have resulted in enormous surge in the number of current

clinical trials, there are still no recognized clinical therapeutic procedures. This review will assess the effect of local and intravenous administration of adipose tissue-derived stem cells on the regeneration of buccal oral epithelium in chemotherapy-treated rats.

MATERIAL AND METHODS

Stem cells

Allogenic PKH26 labelled rat adipose tissue-derived stem cells (ASCs) were obtained from the stem cell research unit at the Biochemistry Department, Faculty of Medicine, Cairo University.

Oral Mucositis (OM) induction:

To achieve immunosuppression induced by anticancer drugs, rats received an intraperitoneal injection with the chemotherapeutic agent 5-FU (Fig. 1) on days 0 and 2 of the experiment at a dose of 50 mg/kg body weight. Subsequently, on day 0, mucosal ulcers were induced by injection of 20% acetic acid (20 μ l) ⁽⁶⁾.



Figure (1) 5- FU

Animals

48 adult male albino rats weighing (200~250gm) were used in this research. At Cairo University animal house, the research animals were raised. Keeping each group in isolated cage at room temperature, with free access to fresh water and ad

libitum for feeding. Maintaining the animals was done according to the typical ethical guidelines of the Institutional Animal Care and Use Committee. The approval of Research Ethical Committee of the Faculty of Dental Medicine for Girls Al-Azhar University was obtained number REC-BI-18-036.

The 48 rats were divided into 4 groups, each group contained 12 rats ⁽⁶⁾.

Group I: served as a control group.

Experimental groups:

Group II: Rats received no treatment ⁽⁶⁾.

Group III: Rats received a single dose (1.5×10^3 - 1.5×10^6) of adipose-derived stem cells given locally once lesions are induced ⁽⁶⁾.

Group IV: Rats received a single dose (1.5×10^3 - 1.5×10^6) of adipose-derived stem cells given by intravenous route ⁽⁶⁾.

From each group, six rats were euthanized after one week and the remaining six rats were euthanized after two weeks. After that, their buccal mucosa was dissected out, fixed in 10% neutral buffered formalin, processed, and soaked in paraffin. For detection of any possible structural changes in mucosa six-micron thick sections were cut to be stained with H&E.

Light microscopic examination

From each group, specimens were obtained and fixed in 10% formol saline for one day. Tap water was used for washing then for dehydration, sequential dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used. With xylene the specimens became clear and soaked in paraffin at 56° C in a hot air oven for one day. Then preparation of Paraffin wax tissue blocks for sectioning at 4 microns thickness by microtome was carried out. On glass slides the targeted tissues were assembled, deparaffinized, stained by H&E stain for examination with the light microscope.

RESULTS

Control group

The epithelium in the control group (Group 1) appeared covered with a thick layer of orthokeratin. Normal sized epithelial cells with normal nuclei were tightly packed together with no intercellular bridge disruption and clear cells appeared scattered between these keratinocytes. Epithelial rete pegs appeared short, broad and irregular. The underlying C.T. appeared normal with no signs of inflammation with the muscle fibers running in different directions.

After one week

- **Group II** showed extensive ulcers accompanied with loss of keratinization in the existing epithelium adjacent to the ulcer area. Granulation tissue with massive inflammatory cell infiltrate (polymorphnuclear cells PMNs) appeared concentrated in the center of the ulcer. Multiple edematous areas and focal necrosis were detected at different levels. High level of muscle degeneration was clear.
- **Group III (direct-injection group):** The ulcer appeared covered with a continuous layer of regenerated epithelium with thin irregular superficial orthokeratin layer. The epithelial rete pegs disappeared and little degeneration was detected at the basal cell layer. The underlying C.T. exhibited low degree of inflammation and muscle fibers were markedly restored.
- **Group IV (intravenous injection group):** The regenerating epithelium failed to cover the ulcer area and appeared thin and irregular with an interrupted layer of superficial parakeratin layer. The underlying CT showed low degree of inflammation and areas of oedema (Fig. 2).

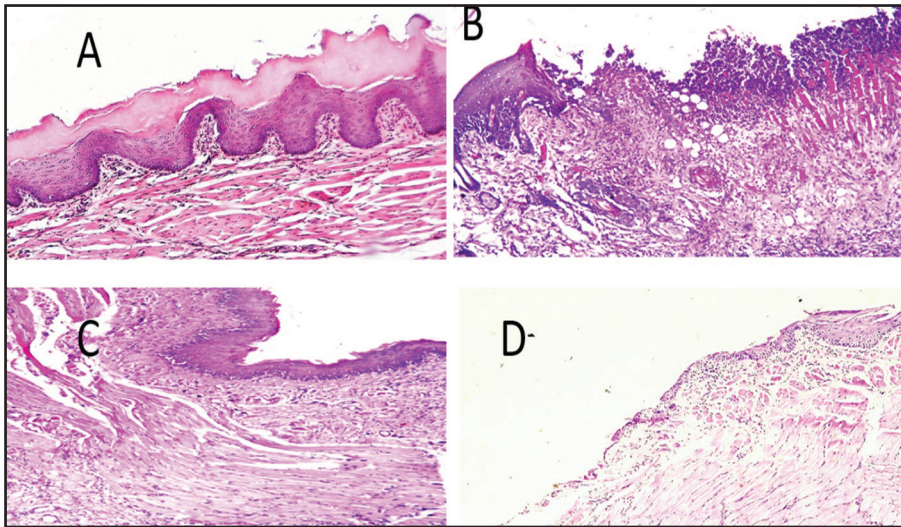


Figure (2) A photomicrographs at first week: [A] Control group (Group I) [B] Group II, [C] Group III and [D] Group IV. (H&E, Orig. Mag x100).

After two weeks

- Group II:** The regenerating epithelium migrated over the ulcer but failed to cover it completely where the defect area was filled with granulation tissue. The regenerated epithelium showed variation in thickness and was covered by a keratin layer. The epithelial rete pegs were few, short and irregular at the periphery and nearly absent near the defect. The basal cell layer was ill defined in some areas. CT showed congested BLVs, areas of oedema with inflammatory cell infiltrate.
- Group III (direct-injection group):** Short, broad epithelial rete pegs grew in the underlying C.T. and the basal cell layer became relatively well organized. The underlying CT showed some engorged BLVs and extensive fibrosis. The muscle layer was properly restored.
- Group IV (intravenous injection group):** Ulcer appeared covered with a continuous layer of regenerated epithelium, no degeneration of basal cell layer, CT shows congested BLVs, areas of oedema, densely packed collagen bundles and the underlying muscles were moderately restored. The thickness of lamina propria was markedly increased in both III & IV groups (Fig. 3).

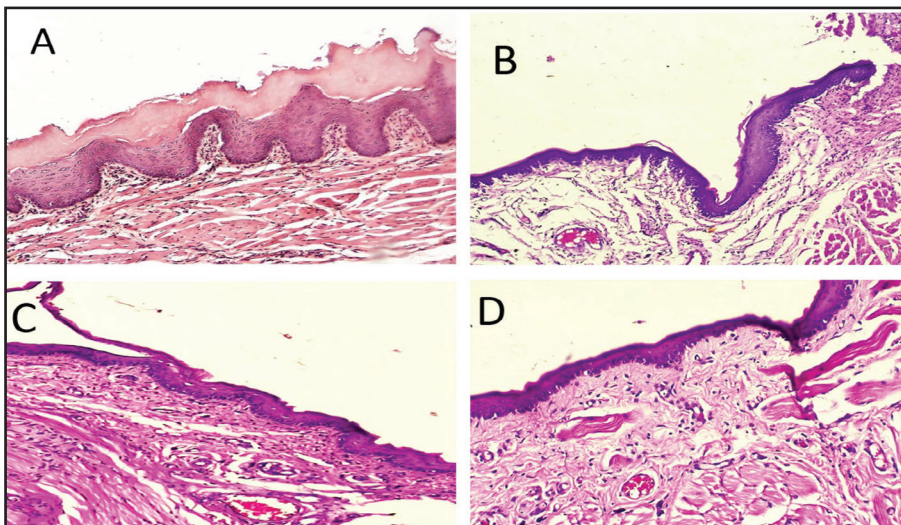


Figure (3) A photomicrographs at second week: [A] Control group (Group I) [B] Group II, [C] Group III and [D] Group IV. (H&E, Orig. Mag x200).

DISCUSSION

Oral ulcers are a common condition with episodic onset of burning pain and ulcer tissue is prone to infection, resulting in inflammation and tissue necrosis. Oral ulcers are thought to be caused by a diversity of influences, including immune disorders, drug stimulation, and bacterial infection, according to several reports ⁽⁷⁾.

The rat was nominated as an animal model in the current research since it is one of the most frequently used research animals with physiological body functions that are nearly matching to those of humans. Additionally, they could be kept, raised, and treated without difficulty. Adult male albino rats were carefully chosen because their hormonal state is steadier than that of female albino rats ⁽⁸⁾.

Numerous studies have publicized that MSCs can stimulate the regeneration of dental tissues such as enamel, dentin, tooth pulp, and PDL, as well as other organs with similar developmental origins such as bone and salivary gland ⁽⁹⁾.

This research aimed to provide a new platform by inspecting the influence of experimentally induced oral ulcers on the adult rat oral mucosa, along with the potential ameliorative outcome of ADSCs and the variance between the direct and intravenous routes of therapy administration.

Mesenchymal stem cells originating from adipose tissue are plentiful in adipose tissue and can be retrieved effortlessly by liposuction. ADSCs, including bone marrow-derived mesenchymal stem cells, can differentiate into osteoblasts, chondrocytes, neurons, and myocytes, among other things ⁽¹⁰⁾.

In this research, the healing power in the group which didn't receive ADSC was much slower than the groups which received ADSC, this supports the fact that the stem cell therapy increases the healing potentials by increasing the blood flow, decreasing inflammation, differentiating into new epithelial cells, the stem cells have been found to have remarkable efficacy in animal experiments and clinical studies of many diseases due to their multi-lin-

eage differentiation potentials, anti-inflammatory, paracrine, and other biological functions ⁽¹¹⁾.

The study revealed that the healing characteristics of an oral ulcer after direct injection of ADSCs in the ulcer were stronger and quicker, the direct injection increases the efficacy of treatment due to direct delivery of stem cells increasing the number of treating cells in the wound while the systemic delivery of stem cells take more time to reach the wound so delayed healing, which was also observed in treating rats diagnosed with brain stroke. The authors demonstrated that direct injection of stem cells had superior results in comparison to systemic administration in their research ⁽¹²⁾.

While systemic administration of MSC has a higher risk of pulmonary embolism and accumulation in healthy organs than local administration, it has been shown to have a higher efficacy ⁽¹³⁾. Other researchers have also shown that local injection of ADSCs is more effective than intravenous injection in treating endometrial disease ⁽¹⁴⁾.

CONCLUSION

Based on the findings, it was determined that 5-FU-induced oral ulcers are linked to degenerative histological changes in the oral epithelium. Injection of ADSCs was found to be a promising method for assisting the regeneration of damaged oral tissues. Furthermore, since the direct injection produced better healing outcomes than the intravenous injection, more research involving other oral tissues and alternative treatment protocols is recommended.

RECOMMENDATIONS

More studies should be carried out in human trials in Stem Cells research to validate human therapy protocols.

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Declaration

No fund was received for this study.

Conflicts of Interest

There was no conflicts of interest in this study.

REFERENCES

- Groeger S and Meyle J. Oral Mucosal Epithelial Cells. *Front. Immunol.* 2019;10:208.
- Longley, D., Harkin, D. & Johnston, P. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer.* 2003;3: 330–8.
- Claudio Pulito, Antonio Cristaudo, Caterina La Porta, Stefano Zapperi, Giovanni Blandino, Aldo Morrone, et al. Oral mucositis: the hidden side of cancer therapy. *J Exp Clin Cancer Res* 2020;39: 210.
- Samsonraj RM, Raghunath M, Nurcombe V, Hui JH, van Wijnen AJ, Cool SM. Concise Review: Multifaceted Characterization of Human Mesenchymal Stem Cells for Use in Regenerative Medicine. *Stem Cells Transl Med.* 2017;12:2173-85.
- Hiroki Ohta, Xiaolan Liu, and Miho Maeda. Autologous adipose mesenchymal stem cell administration in arteriosclerosis and potential for anti-aging application: a retrospective cohort study. *Stem Cell Res Ther.* 2020;11: 538.
- Nascimento-Júnior Braz José do, Brito Lucas de Souza, Barros Walquíria Nunes, Gonçalves Daniela Marques, Matos Luana de Souza, Nascimento Cíntia Reyjane Borges et al. Anti-inflammatory and healing action of oral gel containing borneol monoterpene in chemotherapy-induced mucositis in rats (*Rattus norvegicus*). *Braz J Pharm Sci.* 2017; 53.
- Miao M, Peng M, Xing Z, Liu D. Effect of Shuangjinlian mixture on oral ulcer model in rat. *Saudi J Biol Sci.* 2019;26:790-94.
- Khatab MM, Hulail M, Hegazy A, Mohammed HO. Study of the Toxic Effect of Methimazole on the Cortical Structure of Adult Male Albino Rats and the Ameliorated Effect of Thyroxine. *Z U M J.* 2018;24: 208-19.
- Yang JW, Shin YY, Seo Y, Kim HS. Therapeutic Functions of Stem Cells from Oral Cavity: An Update. *Int J Mol Sci.* 2020;21:4389.
- Tong Liu, Jia Xu, Xun Pan, Zhangfan Ding, Hao Xie, Xiaoyi Wang, Huixu Xie. Advances of adipose-derived mesenchymal stem cells-based biomaterial scaffolds for oral and maxillofacial tissue engineering. *Bioactive Materials.* 2021;6: 2467-78.
- Ronghua Yang, Jingru Wang, Xiaodong Chen, Yan Shi, Julin Xie, "Epidermal Stem Cells in Wound Healing and Regeneration". *Stem Cells International.* 2020; 2020:11.
- Rodríguez-Frutos B, Otero-Ortega L, Gutiérrez-Fernández M, Fuentes B, Ramos-Cejudo J, Díez-Tejedor E. Stem Cell Therapy and Administration Routes After Stroke. *Transl Stroke Res.* 2016;7:378-87.
- Madiyal A, Babu S, Bhat S, Hegde P, Shetty A. Applications of stem cells in dentistry: A review. *Gulhane Med J.* 2018;60:26-29.
- Monsef F, Artimani T, Alizadeh Z, Ramazani M, Solgi G, Yavangi M, Asl SS. Comparison of the regenerative effects of bone marrow/adipose-derived stem cells in the Asherman model following local or systemic administration. *J Assist Reprod Genet.* 2020;37:1861-68.