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Potential Effect of Fibroblast Growth Factor (FGF2) Versus Epidermal Growth Factor (EGF) on Healing of Induced Oral Ulcer in Albino Rats (A Histological and Immunohistochemical Study)

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ABSTRACT

Aim: The aim of the present study was to evaluate the effect of human recombinant Fibroblast growth factor (FGF2) and Epidermal growth factor (EGF) on healing of mechanical induced buccal mucosal ulcers in albino rats. **Materials and Methods** seventy five adult male albino rats (weight 200-250gm) each were used in the study. Rats were divided into 4 groups (group1, group2, group 3 & group 4) : G2 negative control, G3 received FGF, G4 received EGF, then each group was divided into 4 subgroups according to the date of scarification at 1, 4, 7, 10 days postoperatively ulcers were induced by punch biopsy apparatus (5 mm diameter). Growth factor (FGF or EGF) was applied by using microliter syringe, the specimens were processed for histological and immunohistochemical analysis. **Results:** EGF and FGF promoted proliferation of fibroblast, EGF promoted proliferation of keratinocyte. **Conclusion:** Both growth factors accelerate wound healing compared with control group. rh FGF restores collagen production, while rh EGF can induce rapid wound healing by accelerating the proliferation of new epithelial cells.

INTRODUCTION

Traumatic ulcers are the most common inflammatory ulcerative conditions in the oral cavity, and the cheek mucosa is the most traumatized site. Such traumas are caused by poorly adapted prosthesis or occlusal disharmony, tooth crowns or fractured restorations and even by accidental bites while chewing or habits. ⁽¹⁾

KEYWORDS

FGF,
EGF,
oral ulcers,
wound healing.

Paper extracted from master thesis entitled "Potential Effect of Fibroblast Growth Factor (FGF2) Versus Epidermal Growth Factor (EGF) on Healing of Induced Oral Ulcer in Albino Rats (A Histological Study and immunohistochemical)"

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The healing of any ulcers included the granulation formation and re-epithelization, it would be accelerated obviously by adding exogenous growth factor. EGF and bFGF are important modulators of wound healing; EGF promotes the proliferation of keratinocytes, and re-epithelialization. On the other hand, bFGF is potent mitogen for mesodermal derived cells, angiogenesis and the formation of granulation tissue. ⁽²⁾

The growth factor is a special kind of factors which can stimulate the proliferation of target cells, increase the synthesis of extracellular matrix and promote the wound healing. It's an important bioactive polypeptide that participates in wound healing of trauma and large-area burn. With the development of modern genetic engineering technology, the growth factor has been batch to obtained and used widely in wound healing. ⁽³⁾

FGFs are multifunctional regulatory peptides with a great impact on studies of tumor genesis, cardiovascular disease, and repair of tissue injury, neurobiology and embryonic development. They are responsible for critical functions in wound healing, tissue repair, angiogenesis, and homeostatic regulation. ⁽⁴⁾

EGF is mitogen for epithelial, mesothelium, and endothelial cells and has been shown to accelerate re-epithelialization, increase proliferation and improve over all wound healing. Re-epithelialization involves migration of epithelial cell from ulcer margin onto the granulation tissue to re-epithelialize the ulcer base and restore epithelial continuity which activated by binding of EGF to its receptor. ⁽⁵⁾

MATERIALS AND METHODS

Seventy five adult male albino rats (weight 200-250gm) each were used in the study, the animals obtained from Theodor bilharzias (Giza, Egypt). Rats are caged under normal laboratory condition, and kept in standard cage at room temperature of 20-25° they were acclimated for 10 days and provided with standard diet and allowed free access of water.

Animals will follow the rules and regulation of the animal experimental studies approved by Ethical Committee including their facilities diet and method of scarification.

Rats were divided into 2 main groups (control, experimental) and experimental divided into 3 groups (G2: negative control, G3: FGF group, G4: EGF group) then each group divided into 4 subgroups according to the date of scarification at 1,4,7, 10 days postoperatively.

The rats were anesthetized with intra peritoneal Xylazine hydrochloride 0.1-0.2mg/kg IM for 45 minutes, ketamine 10-15 mg/kg IM.⁽⁶⁾ Then, ulcers were induced by punch biopsy apparatus (5 mm diameter). a 1mm-thickening of the buccal mucosa producing uniform circular cutting on the right Buccal mucosa of the cheek.

At the time of surgery 1μg of FGF & EGF (provided from Sigma –Aldrich pharmaceutical company) dissolved in 100 μl of phosphate buffered saline, was applied by using microliter syringe. Injection was done into sub mucosa adjacent to the edge of the mucosal defect at 4 selected sites. ⁽⁷⁾

Animals were deprived from food and water for 30 minute after application. The animals in the experimental groups (G2, G3, G4) were sacrificed at different intervals, at D 1, 4, 7 and 10. The collected specimens were proceeding for light microscopy (L.M) and immunohistochemical examination (IHC).

Histology and immunohistochemical

Tissue specimen were excised and immersed in 4% paraformaldehyde in 0.1 PBS at room temperature. Then the specimens were transferred to 0.1 M PBS containing 30% sucrose. Cryostat section (10 to 20) μm thick were cut and mounted onto glass slide. The section were stained with H.&E. a rabbit anti- PCNA polyclonal antibody were used for immunohistochemical.⁽⁸⁾

RESULTS

At day 4, the morphology of the newly formed epithelium that appeared migrating downward and lateral overlying connective the same in both (FGF, EGF) group (Fig.1:C,E). The defect appeared smaller than the control group (Fig.1: A).

PCNA was detected as a brown chromogen staining of tissue components expressing a positive PCNA monoclonal antibody

At day 7, wound was closed completely, but epithelium in FGF group appeared thin than the epithelium in EGF that appeared thick with retepage.

On the other hand angiogenesis and mature granulation tissue in FGF group (Fig.2: c), while in EGF group granulation tissue was less matured (Fig.2: e). The number and distribution of proliferating cells were analyzed by use of immunostaining against proliferating cell nuclear antigen (PCNA). In control group, distribution of PCNA positive cells located mostly in basal cell (Fig. 1:B ,2:b).

Number of PCNA positive cells was increased in epithelium and connective tissue of FGF and EGF group. At day 7 number of PCNA positive cells was increased in FGF group in both epithelium and connective tissue more than EGF group (Fig. 2:d,f).

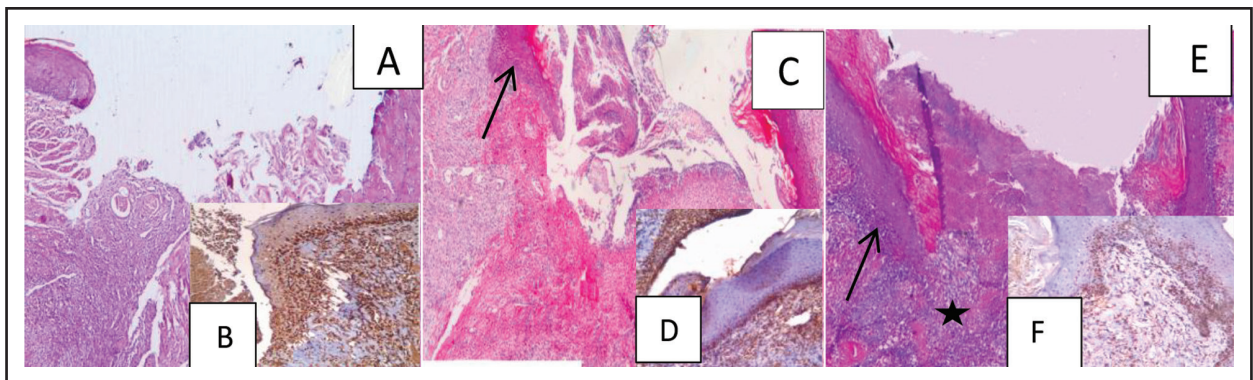


Fig. (1) Photomicrographs of rat buccal mucosa at day 4. A) G2 H.&E., B) G2 PCNA, C) FGF group H.&E., D) FGF group PCNA, E) EGF group H.&E., F) EGF group PCNA. Arrow: epithelium, star: inflammatory cells.

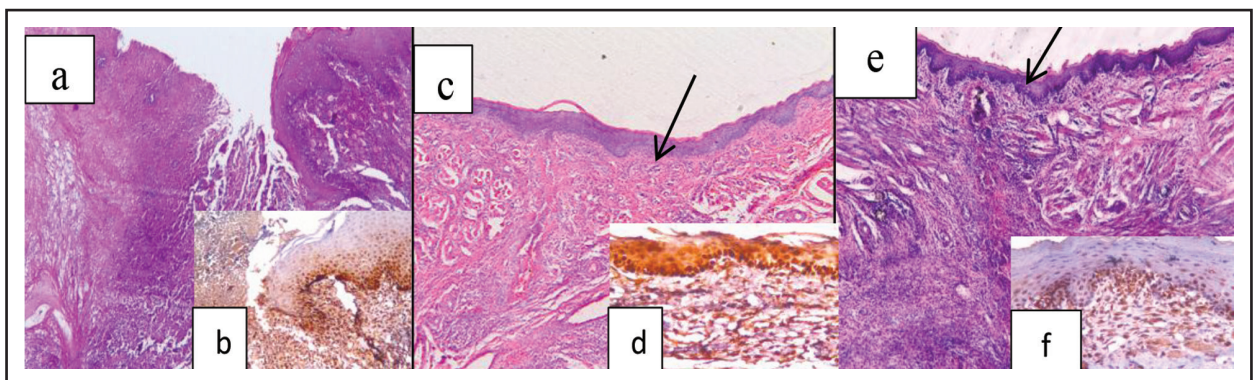


Fig. (2) Photomicrographs at day 7 :a) G2 H.&E., b)G2 PCNA, c) FGF group H.&E., d) FGF group PCNA, e) EGF group H.&E., f) EGF group PCNA, arrow: healed ulcer.

DISCUSSION

Ulcers are the most common inflammatory conditions in the oral cavity, and the cheek mucosa is the most traumatized site. ⁽⁹⁾Rats were used as experimental animal which represented a perfect model for examination of oral tissues and their associated structure this examination wouldn't be easily done in human due to ethical reasons. Also, there was similarity between the oral mucosa of rats and humans. Furthermore, rats had some advantage: the low cost, the easy manipulation, maintenance in controlled environmental and sanitary conditions including special diets. ⁽¹⁰⁾

In the present study, oral mucosal ulcers were created by a 1mm-thickening of the buccal mucosa with a punch biopsy apparatus (5 mm diameter) that method was produce uniform ulcer with fixed depth and width. Application of that apparatus was applicable, fast during operation. So, the duration of anesthesia was reduced. in the present study, a dose of both growth factors per rat was $1\mu\text{g} / 100\mu\text{l}$ taken once per day, this corresponding to the previous study ⁽²⁾

At day 4 in negative control group the defect still wide, the same finding of epithelium and connective tissue at day (1) appeared here but with slight increase associated with loss of normal architecture of muscle, was in agreement with other study.⁽³⁾

In FGF, EGF group the defect of wound was decreased than the control group, the epithelium migrate lateral and downward in both group trying to close the defect. Regard to connective tissue, it was found that in FGF group: the connective tissue filled with much, large, spindle fibroblast this may be FGF is mitogen for fibroblastic cells according to other study ⁽¹¹⁾ In EGF group the connective tissue filled with heavy and dense inflammatory cells with areas of hyalinized collagen.

At day 7 in control group the defect slightly decreased than before and the epithelium began to migrate lateral and downward over the connective

tissue these forgoing feature showed at day 4 in FGF & EGF (treated groups). Connective tissue showed chronic inflammatory reaction. ⁽³⁾

In both (FGF, EGF) the defect was healed completely but with different thickness, in FGF group the wound area was healed completely with thin orthokeratinized stratified squamous, while in EGF group the wound area was healed completely with orthokeratinized stratified squamous epithelium nearly of normal thickness with rete pegs formation.

Connective tissue in both treated group (FGF, EGF) was different, since in FGF group the connective tissue filled with new blood vessels formation (angiogenesis) according to the previous study ⁽¹²⁾ it was found that bFGF has most effective potential activity for angiogenesis. It has been observed in animal studies that where bFGF had been applied at the early phase of the wound healing process, many newly formed capillaries were observed around that area. Furthermore, mature collagen fibers formed in the newly formed granulation tissue this indicate faster maturation of collagen in (FGF group) which not observed in the control group, this corresponding with the previous study ⁽⁷⁾

In EGF group, granulation tissue was less matured than FGF group. So ,according to the previous study ⁽²⁾ where EGF and bFGF stimulated the ulcer healing of rabbit oral mucosa, but bFGF could be more effective than EGF in accelerating granulation tissue formation and its replacement by connective tissue.

Proliferating cell nuclear antigen (PCNA) was expressed throughout the cell cycle and its concentration was increased further in the S-phase. ⁽¹³⁾ It have been demonstrated that the capacity to accelerate the ulcer healing process depends on many factors, such as fibroblast growth factor, vascular-endothelial growth factor, and epidermal growth factor that stimulate angiogenesis and cell proliferation.

It was noted that both group (FGF, EGF) showed increase in PCNA immunoreactive cells than the control group at day (7) but FGF showed slight increase in number of PCNA positive cells than EGF because of PCNA-positive cells were not only localized in the epithelium but also distributed in the connective tissue. This result indicates that bFGF not only may accelerate wound healing through re-epithelialization but also may affect wide range of cells in connective tissue. ⁽¹⁴⁾

CONCLUSIONS

Both growth factors accelerate wound healing compared with control group, Exogenous administration of rh FGF restores collagen production and promotes the wound healing, Exogenous administration of rh EGF can induce rapid wound healing by accelerating the proliferation of new epithelial cells.

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