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Montelukast Tackled Epidermal Growth Factor Expression in Mouth Ulcer Model in Rats

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Abstract

Mouth cleansing is part of everyday activities to avoid oro-dental diseases. Despite that mouth, diseases are reported as a common cause for seeking medical help. Once this happens, oral ulceration continues for days or weeks depending on the personal oral hygiene. The mouth cavity contains different types of bacteria as a part of normal flora. Tissue layers are protected by the mucosa layer, and torn mucosa due to ulceration will expose tissue to the external environment resulting in infection and inflammation. The present study aimed at identifying the anti-inflammatory effects of montelukast via follow-up of their wound healing properties over different time points, compared to control. To do so, a rat mouth ulcer model was used by categorizing rats into 2 groups of 12 in each; montelukast versus the control group. Histology and Deposition of EGF as a healing marker detected by immunohistochemistry technique were used to compare montelukast versus control. The outcome confirmed that montelukast accelerated the healing process confirmed by more EGF expression at the salivary glands and hence quicker healing resulting in reduced EGF expression at day 3 or 7 compared to the control group. In conclusion, montelukast has anti-inflammatory and wound healing properties which could make it a good candidate for future topical preparation formulation for burns and wounds.

Keywords: Montelukast, Healing, Oral Ulcer, Rats, EGF.

Introduction

A group of newly introduced drugs for the treatment of asthma is called leukotriene antagonists; as a synthesis inhibitor via lipoxygenase blockade (e.g. zileuton) reducing production or receptor blockade (e.g. Zafirlukast and Montelukast). The latter achieves better therapeutic action compared to the former. Montelukast is widely used due to its one-daily dose, long action, and better side effect profile compared to zafirlukast; a member of the same group (Dengiz *et al.*, 2007, Abdelhady *et al.*, 2021). These commercially available medications are currently in clinical therapy for asthmatic patients, alongside other therapeutic actions on the respiratory system including bronchospasm, and allergic rhinitis (Tesfaye *et al.*, 2021).

Mouth lesion represents one of the challenging oral diseases which urgent medical seeks (Rezazadeh *et al.*, 2021, Abid and Naser, 2021), due to its interference with the patient's daily activity, including eating, chewing, talking, and liquid drinking; which necessitate accelerating the healing process (Wu *et al.*, 2021). Treatment of oral lesions is perturbed due to several factors, including the presence of a huge number of microorganisms, the friction between walls of the mouth, and the wet nature of oral mucosa. Therefore, attention has been paid to finding new treatment modalities which might accelerate wound healing including searching for new treatment remedies to interfere with lesions and accelerate the healing

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process. This study was designed to use montelukast as antiulcer agents using in vivo induced ulcer model via determining the immunohistochemical expression of EGF (Enaia *et al.*, 2011).

Methodology

A total of 24 healthy rats (age 2-3 months; weight 250-350g) were kindly provided by an animal house in the College of Veterinary Medicine at the University of Mosul. Place, food, water drink, temperature, and light/dark cycles were all fixed during the study period according to standard animal care protocols.

These 24 rats were divided into two groups (12 each; montelukast versus control group) and mouth ulcers were induced using acetic acid. Firstly the animals were sedated using the standard anaesthesia method (intraperitoneal injection of a mixture of xylazine 5 mg/kg and ketamine hydrochloride 50 mg/kg). After sedating the animals, the mucosa at the tongue's dorsal surface was exposed to acetic acid (70%) for 2 minutes resulting in white discolouration of mucosa which indicates mouth lesion, which has been confirmed two days after (Idrus *et al.*, 2019, Miao *et al.*, 2019). These 24 rats were treated with either distilled water or 20mg/kg/day montelukast (Merck, UK) orally. On day3 six animals were sacrificed from each group and on day 7 the remaining animals were sacrificed

Wound sections were collected and exposed 24 hours to paraffin (10%) for fixation (Al-Allaf and Al-Ashoo, 2021). Samples were then treated with a series of alcohol concentrations for dehydration and finally dipped into xylol impregnated with thawed paraffin at 60°C. Samples were then banded into paraffin-forming blocks. The samples were transferred to a microtome for slice (5Mm) formation to be ready for immunohistochemistry study (Mammdoh *et al.*, 2020).

Results

The results of analysis of the slides of tongue lesion in the control group have shown a very high expression (score 3) by day 3 and continued same thereafter over further time points at day 7 and even the slides showed similar EGF expression comparable to positive control. On the other hand, the lower expression has been noticed in the montelukast treated group on day 3 (score 1) and day 7 (score 2) see Figure 1.

The results of analysis of the slides of salivary glands in the control group has shown a very low expression (score 1) by day 3 and continued same thereafter over further time points at day 7 and even the slides showed similar EGF expression comparable to negative control. On the other hand, a higher expression has been noticed in the montelukast treated group on day 3 (score 3) and day 7 (score 2) see Figure 2.

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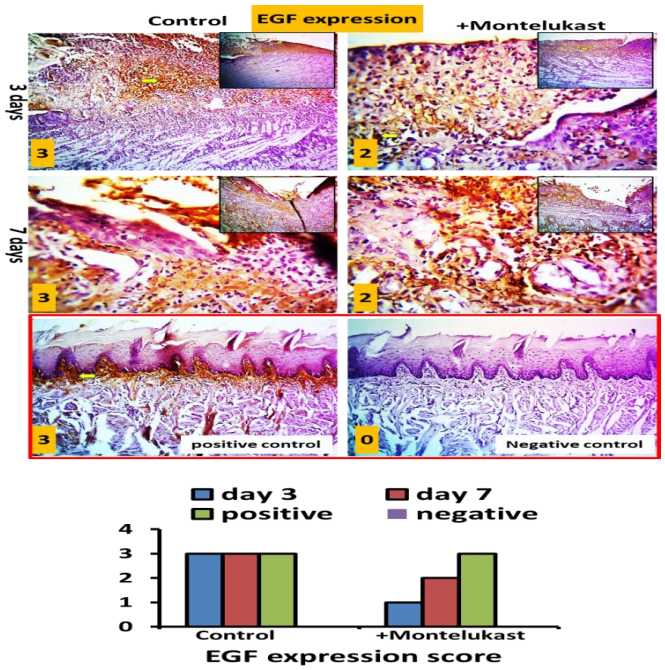

Figure 1. Representative images for EGF expression by ulcer induced in tongue tissue on day 3 and day 7 in Montelukast treated versus the treatment-free control group. Montelukast has greatly modified EGF expression.

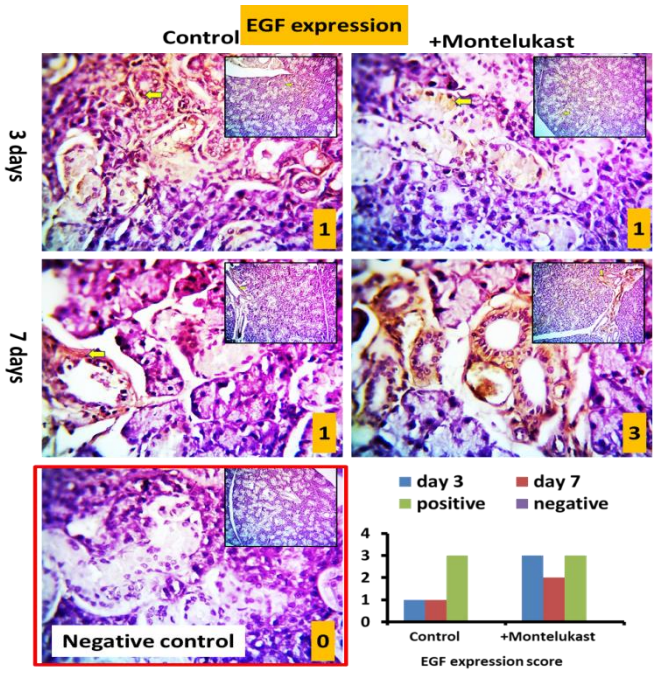

Figure 2. Representative images for EGF expression in Salivary Glands on day 3 and day 7 in Montelukast treated versus the treatment-free control group. Montelukast has greatly induced EGF expression scores.

Discussions

The present study has confirmed that montelukast notably reduced the concentration of epidermal growth factors at the tissue level in chemically induced mouth ulcers in rats model

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based on tongue dorsal surface injury model using immunohistochemistry detection technique. These results confirmed that montelukast greatly accelerates wound healing compared to control. The concept based on the montelukast blocks neutrophils chemotaxis via inhibiting leukotriene chemotactic receptors which do involve in neutrophil rolling and adhesion(Schmitt-Grohé and Zielen, 2005), thereby inhibiting the inflammatory reaction of mouth lesions. A separate study conducted by Sener et al. reported that the acceleration of healing induced by montelukast owed to its inhibitory effects on myeloperoxidase activity and reduction of oxidative stress (Şener *et al.*, 2005). Montelukast characterizes by its anti-inflammatory Nonetheless, we are looking for additional factors is EGF which is assumed to be regulated by montelukast (Dengiz *et al.*, 2007). According to manufacturer instruction, the antibody (Abbexa132097) supplied by the kit measured the free form of the EGF, hence, results has shown that montelukast has increased the EGF expression by salivary glands on day 7 (figure 2). Correspondingly, the free form was lower at site of lesion in tongue at day 3 or days 7 compared to control groups regarding the same time points. This finding potentially indicates that there has been higher consumption of EGF at site of injury in presence of montelukast.

Montelukast has been used to mitigate indomethacin-induced gastric ulcers in rats. The results have confirmed that montelukast has reduced microscopically gastric lesions to a greater extent than that of lansoprazole, famotidine or ranitidine (Halici *et al.*, 2005, Bayir *et al.*, 2006, Odabasoglu *et al.*, 2006). Additionally, montelukast has been shown to decrease the catalase activity induced by indomethacin, proposing their usefulness in ulceration (Dengiz *et al.*, 2007). Moreover, montelukast provides mucosal protection via activation of glutathione enzyme (Hayes and Pulford, 1995), nonetheless, the healing process also involves several growth factors including EGF (Kwon *et al.*, 2006) especially in mouth ulceration because it is secreted by salivary glands (COHEN, 1962).

A pilot study conducted on recurrent aphthous stomatitis by Femiano *et al.*, 2010 confirmed that montelukast induced comparable improvement compared to prednisolone; provided that montelukast has lower side effects compared to prednisolone. Similarly, a case study conducted by Aquino and Jamora, 2020 confirmed that montelukast improved recurrent aphthous stomatitis when patients were transferred to montelukast for 5 months. These studies have ignored the role of growth factors which could be the refereed parameters in the scenario of mouth lesions. In the oral cavity, these growth factors are released mainly by submandibular glands (Tebar *et al.*, 2000). In an interventional study conducted in 2017 on patients with minor recurrent mouth ulcer stomatitis using montelukast as a tested drug versus vitamin B complex® as a control group, the outcome after 2 months of therapy revealed that montelukast has reduced the pain, reduced the number of ulcers, and duration of healing reduced (Trial, 2019).

The small sample size and the dose design need further studies to identify the best dose alongside the preparation of topical formulation which could produce a better effect than systemic ones. Our future direction will focus on statins (e.g. rosuvastatin and atorvastatin) which has been found to have additional pleiotropic effects (Almukhtar *et al.*,2020, Almukhtar *et al.*, 2021, Almukhtar *et al.*, 2022) alongside their hyperlipidemic (Althanoon *et al.*, 2020) effects which might be helpful in producing similar effects

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Conclusion

The mouth lesion involves numerous factors leading to the betterment of the lesion to cure the ulcer, including EGF. This factor might increase due to different actions and drugs. Montelukast improves its secretion and the lesion response to the therapy.

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