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Original article

Effect of a combination of mometasone furoate, levofloxacin, and retinyl palmitate with an in situ gel-forming nasal delivery system on nasal mucosa damage repair in an experimental rabbit model

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ABSTRACT

Background: In this study a combination of Mometasone Furoate (MF) + Levofloxacin hemihydrate (LH) + Retinyl palmitate (RP) with an in situ gel-forming delivery system was evaluated at different stages of nasal mucosal damage repair in a rabbit maxillary sinus model.
Methods: In this study, 28 rabbits were included and assigned randomly to four groups. In all rabbits, a standard ostium was opened in the medial wall of the maxillary sinus by using a drill. Two different subsequently prepared gels with an in situ gel-forming delivery system were used. Of these 14 nasal cavities, combination 1 (active combination) was applied daily to 5, combination 2 (placebo) to 5, while 4 did not receive any pharmaceutical treatment. The diameter of the ostium was measured. Histopathological assessment was performed.
Results: After 2, 3 and 4 weeks, the ostium diameter was significantly wider in the group where gel 1 had been applied compared to both the placebo group and control group. In the group treated with gel 1, after 2, 3 and 4 weeks the presence of superficial cilia was significantly greater, surface epithelium significantly less. In the 4th week, histologic scores for fibroblastic proliferation and vascular proliferation in the group treated with gel 1 were better than in either the control group or the placebo group. With gel 1, chronic inflammation parameters were also significantly lower than in the other groups.
Conclusion: The MF + LH + RP mixture with an in situ gel-forming nasal delivery system applied for wound healing after FESS prevents the formation of stenosis and is favorable for proper wound healing.

1. Introduction

For chronic sinusitis not responding to medical treatment, functional endoscopic sinus surgery (FESS) is frequently performed. Despite advanced surgical techniques and instruments, major complications such as postoperative scarring, ostium stenosis or adhesions between the medial concha and the lateral nasal wall can arise, causing severe problems by disrupting the mucociliary drainage of the sinuses [1–3]. To guarantee successful surgical interventions, maximum postoperative wound healing needs to be achieved. Nasal packings (tamponage inside the nose) and wound debridement applied to prevent these complications have been shown to have significant efficacy in the prevention of ostium stenoses and adhesions. Yet as foreign objects staying inside the body, they promote scar formation [4–6].

FESS allows the restoration of natural mucus clearance pathways with a minimally invasive intervention [7]. As this technique became popular, the importance given to mucosal protection increased, given that bone tissue exposed by mucosa ablation can develop chronic inflammation leading to osteitis [8]. In addition, abnormal mucosal healing in these regions can result in insufficient mucosal clearance [9]. However, an unchanging etiological source shows that disrupted mucociliary clearance and immobile sinonasal secretions are a principal pathophysiological outcome [10]. It also shows how important post-FESS wound healing is. Wound healing has been researched before in other tissues (gingiva, skin) [11]. Wound healing phases vary between tissues. Generally, they consist of clotting, inflammation, matrix deposition and remodeling, cell proliferation, and maturation [12]. This process is organized by a wide range of contributors, such as growth

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factors, cytokines, and proteases [13]. Local wound healing problems may also affect normal mucosal structure. Thus, the most important factor for the success of surgery is the quality of healing in the mucosa.

To improve nasal mucosal healing post-FESS, systemic antibiotic therapy, systemic corticosteroid, topical steroid sprays, and physiological saline sprays (hypotonic, isotonic, and hypertonic) are in use. These methods may not be sufficiently effective individually, and when used in combination can produce side effects and even then not lead to a complete recovery of the nasal mucosa. To this day, no drug application to minimize post-FESS formation of scar tissue and adhesions and to quickly restore normal sinus functions through improved wound healing has been found.

A drug to improve nasal mucosal healing needs to feature the following four important characteristics [14]:

1. The substance needs to be applicable in a suitable manner
2. In order to be absorbed in the sinus wound, it needs to remain there for a sufficient period
3. It needs to be biocompatible
4. Wound healing needs to be achieved without scarring

The aim of our study is to develop a product that will improve the healing of the newly formed ostium after FESS and improve the mucus healing. In this respect, we aim to increase patient satisfaction with surgical success. For this purpose, in our study we developed a new mucoadhesive nasal drug delivery system, which is liquid at room temperature but on the nasal mucosa converts into gel form, using fast gelating poloxamer 407 and mucoadhesive carbopol 974P NF polymers containing mometasone furoate (MF), levofloxacin hemihydrate (LH), and retinyl palmitate (RP) as active ingredients.

With this combination, we assessed the various stages of nasal mucosal healing in a rabbit maxillary sinus model.

2. Material and methods

2.1. Study design

Ethics approval was obtained from the local experimental animal ethics committee (Number 14/138). Our work is a prospective, randomized, placebo-controlled, double-blind experimental study. For the experiments, 28 white, male New Zealand rabbits weighing between 2 and 4 kg were used. It is accepted that New Zealand rabbits are a suitable animal model for the assessment of nasal mucosal healing in experimental sinusitis models [15–18]. Despite the known physiological differences between humans and rabbits, no more suitable alternatives have been found to date [19].

The animals were divided into 4 groups, conforming with the numbers and times specified in the literature [20,21]. Each group was divided into three subgroups. The groups were sacrificed sequentially at a distance of one week in order to assess the width of the ostium and the recovery of the wound. Thus, within each group placebo and control (negative control) were assessed. For this purpose, in each group the right nasal cavity of five rabbits was used as the study group and the left cavity as placebo group. In two more rabbits, both nasal cavities served as control group (Fig. 1).

3. Composition of mometasone furoate (MF) + levofloxacin hemihydrate (LP) + retinyl palmitate (RP) mixture with an in situ gel-forming delivery system

3.1. A. Materials used in the preparation of the combination

Mometasone furoate (Sigma Aldrich, Germany), Levofloxacin hemihydrate (Sigma Aldrich, Germany), Carbopol 974P NF (Lubrizol, Belgium), Dexpanthenol (Fluka, Germany), Benzalkonium chloride (Fluka, Germany), Pluronic® F-127 (BASF, Germany), Retinyl palmitate (Sigma Aldrich, Germany), Polyethylene glycol 400 (Tekkim, Turkey). All other chemicals used were of analytical grade.

For the study, two combinations were prepared. Combination 1 contained active ingredients plus additional substances (components to achieve in situ gelation). Combination 2 (placebo) contained additional substances only (Table 1).

3.1.1. Combination 1 (active)

Mometasone furoate (MF), Levofloxacin hemihydrate (LH), Retinyl palmitate (RP) + ***Combination 2*** (Carbopol 974P NF, PF127, PEG 400, Dexpanthenol (as a humidifier), Sodium chloride, Benzalkonium chloride, Citric acid solution (50% m/v), distilled water)

3.1.2. Combination 2(placebo)

Carbopol 974P NF, PF127, PEG 400, Dexpanthenol, Sodium chloride, Benzalkonium chloride, Citric acid solution (50% m/v), distilled water

3.2. B. Preparation and optimization of heat-gelating PF 127 gels

PF 127 gels without and with active ingredients were prepared according to the cold preparation method described by Schmolka [22]. Initially, in order to optimize the formulation, gels without drugs were prepared with various concentrations of PF 127 and the gelation temperatures were assessed. The series with an optimized PF 127 concentration was used in further studies to research the effect of the mucoadhesive polymer on the gelation temperature. The mucoadhesive polymer Carbopol 974P NF was prepared in two different concentrations (0.25% and 0.5%).

According to the chosen cold preparation method, the drug-laden formulations were prepared stirring MF, LH, RP, PEG 400, Carbopol 974P NF and the other ingredients at room temperature into the appropriate quantity of distilled water. After cooling the dispersion in a refrigerator to 4 °C, PF 127 was slowly added to the dispersion with continuous stirring with a magnetic stirrer. The final dispersion was kept in the refrigerator until it became a clear solution [22]. Finally, the formulations were calibrated to the required volume using distilled water. The transparency, pH, gelation temperature and viscosity of the final formulations were assessed.

3.3. C. Assessment of the Formulations

3.3.1. Transparency

Transparency of the formulations was assessed visually in front of a black and white background, using the categories turbid (+), clear (++), very clear (glass-clear) (+++) [23].

3.3.2. pH

To determine the pH values of the formulations, 1 ml from each formulation was transferred into a 10 ml beaker and diluted with distilled water to 10 ml. The pH values of the resulting solutions were measured with a pH meter (WTW inolab pH 720) [23].

3.3.3. Determination of the solution-gel-transition temperature (T sol-gel)

Gelation temperature of the aqueous solution containing PF 127 was determined in the following way: 10 ml solution was transferred into a 20 ml transparent beaker with a magnetic stirring bar. At a stirring speed of 100 rpm, the temperature on the heated stirrer was increased by 1 °C/min. The temperature at which the stirring bar stopped turning was determined as the gelation temperature [23].

3.3.4. Determination of the formulations' viscosity

The static rheological characteristics of the preparations were determined using a rotational viscometer (Brookfield DV-II viscometer, Brookfield Engineering Laboratories Inc., MA, USA). The viscosity measures were taken at room temperature and at 34 °C, using a