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Development of an intranasal formulation containing indomethacin and xylometazoline for rhinosinusitis treatment

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Abstract. – OBJECTIVE: Use of the nasal route of drug administration dates back many years and is used both to achieve topical treatments and to allow systemic absorption. The objective was to develop a formulation with novel features which enhance prolonged contact with the nasal and sinusal lining, since this should increase any therapeutic benefit. The anti-inflammatory drug selected was indomethacin, which was combined with xylometazoline, an effective nasal decongestant agent.

MATERIALS AND METHODS: 28 Sprague-Dawley rats were used. They were then allocated at random to one of the four groups of equal size. All rats received a nasal application of 50mL of the platelet-activating factor solution at a concentration of 16 μg/mL and had induced rhinosinusitis. Indomethacin or xylometazoline HCl or both were dissolved in the oily phase of the solution and then a magnetic stirrer was used to homogenize the solution for 60 min at room temperature. All the O/W solutions exhibited stability and remained at neutral pH for the entire duration of the experiment. The only intervention was application of inactive 0.9% saline in group 1. The intervention was nasal application of xylometazoline and indomethacin in the combined formulation in group. The intervention was nasal application of xylometazoline only in group 3. The intervention was nasal application of indomethacin only in group 4.

RESULTS: For the animals in group 1 (the controls), the mucosa had sustained a significant level of damage and the vessels were highly congested. Inflammatory cells were extensively infiltrating the mucosa. (Figure 1 – A1, 2, 3). In group 2, by contrast, the vessels were hardly congested and there were very few infiltrates. The epithelium appeared completely intact (Figure 1 – B1, 2, 3). Furthermore, when groups 1 and 2 were compared in terms of congested vessels, inflammatory cellular infiltrates and injury to the epithelium, the differences reached statistical significance, with p-values of <0.01, >0.001 and <0.001, respectively. Comparison of groups 2 and 4 with the control group also revealed statistically significant differences in terms of cellular infiltrates ($p<0.001$) and damage to the epithelium ($p<0.001$). For the degree of congestion of the vessels, however, the difference between groups was not at the level of statistical significance ($p<0.071$).

Groups 3 and 4 differed at a statistically significant level in terms of degree of congested vessels, cellular infiltrates, and damage to the epithelium ($p<0.025$ and $p<0.001$). The sections from rats in groups 2 and 3 had a lower degree of congested vessels, which may be due to the actions of xylometazoline.

CONCLUSIONS: In the future, topically applied intranasal NSAIDs will be valuable formulations. Innovative types of formulation, such as those demonstrating thixotropic behavior, permit the agent to remain in prolonged contact with the nasal and sinusal lining. Alongside increased efficacy, these preparations will also improve the side effect profile of NSAIDs, largely eliminating systemic effects.

Key Words:
Xylometazoline, Indomethacin, Intranasal Formulation.

Introduction

Rhinosinusitis occurs with high frequency. Its characteristic feature is inflammation of the mucosal lining of the nasal interior and paranasal sinuses. Since inflammation is a feature shared by both acute and chronic forms of the condition, treatment approaches should normally consider

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anti-inflammatory agents the most efficacious choice, as well as antibiotics if a specific bacterial pathogen is implicated. The fact that treatment guidelines seldom mention the use of anti-inflammatory drugs is surprising but may reflect concerns about their associated adverse effects or problems stemming from non-standardisation[1-3].

Antibiotics play an undoubted major role in managing rhinosinusitis, acute or chronic, but there are several concerns related to their use in this condition. Firstly, researchers still disagree on precisely how efficacious antibiotic treatment of acute bacterial rhinosinusitis actually is[4]. Then there is the uncomfortable situation that antibiotic therapy in chronic rhinosinusitis, whilst endorsed by evidence-based guidelines, depends on a relatively slender and controversial evidence base. It has been proposed recently that both acute rhinosinusitis (ARS) and chronic rhinosinusitis (CRS) should be treated by systemic administration of steroids, since these agents suppress the inflammatory response[4,5].

Indomethacin is a non-steroidal anti-inflammatory drug with high potency and with numerous clinical applications. The main useful actions of this drug are in suppressing pyrexia, inflammation, and pain. Indomethacin received marketing approval in the USA in March 2010. There are several licensed indications[6]. In common with NSAIDs generally, its use may be limited by adverse effects on the gut. However, if an ideal topical nasal preparation existed, it would combine the desirable effects of the drug with a low incidence of side effects.

One invariable feature of nasal infections is oedema of the mucosa. Decongestant agents help to reduce nasal congestion, improving the patency of the nasal airway and permitting resolution of the infection. It is important for the efficacy of topical nasal agents that they remain in contact with the nasal lining for a prolonged period.

Use of the nasal route of drug administration dates back many years and is used both to achieve topical treatments and to allow systemic absorption. The list of agents employing this route include antibiotics, vasoconstrictive drugs used to reduce nasal congestion, topical antihistamines, and local anesthetics. Despite the fact that nasal administration is often possible, for practical reasons it may not be the preferred choice. The development of agents in a suitable format for nasal administration calls for consideration of multiple aspects. When a nasal formulation is being developed, specific pharmaceutical aspects of the agents need to be balanced against clinical requirements and the need to satisfy regulatory requirements. With this in mind, we aim here to formulate a product suitable for treating sinusitis and containing both an anti-inflammatory active agent and a decongestant. The formulation is first evaluated from an *in vitro* perspective, then shown to be efficacious *in vivo*, using an animal model of sinusitis[7,8].

The objective was to develop a formulation with novel features which enhance prolonged contact with the nasal and sinusal lining, since this should increase any therapeutic benefit. The anti-inflammatory drug selected was indomethacin, which was combined with xylometazoline, an effective nasal decongestant agent[9].

Materials and Methods

Preparation of the Animal Model

For the experiment 28 Sprague-Dawley rats were used, with a body mass of between 250 and 350 g. The average body mass was 300 g. Rats were chosen that appeared completely free of signs of a respiratory tract infection. These healthy animals were then allocated at random to one of four groups of equal size. The rats were maintained in a controlled atmosphere with constant humidity and temperature. They were free to feed and drink as required and were exposed to alternating 12-hour cycles of light and darkness. Ethical approval for the research, including surgical procedures, was granted by decree number 172 of the Animal Ethics Committee at Eskisehir Osmangazi University.

Pharmaceutical Agents

Indomethacin was obtained from Deva Pharmaceutical A.S. (Istanbul, Turkey). Xylometazoline HCl was obtained from Berko Pharmaceuticals (Istanbul, Turkey). All chemicals used in the experiment were of analytical grade.

Preparation of Nasal Formulations

An Oil-in-water (O/W) emulsion of Black Cumin oil was produced in which Tween 80 and Span 80 acted as emulsifiers. The aqueous phase of the solution contained sodium carbonate (2.5%). Indomethacin and xylometazoline hydrochloride were prepared as O/W emulsions, both separately and combined. Indomethacin or xylometazoline HCl or both were dissolved in the oily phase of the solution and then a magnetic stirrer was used to homogenize the solution for 60 min at room temperature. All the O/W solutions exhibited stability and remained at neutral pH for the entire duration of the experiment[7,8].

The following different formulations were used:
- F-1 was the placebo. This solution contained 0.9% sodium chloride only, without any active ingredient.
- F-2 contained both indomethacin and xylometazoline HCl, at a concentration of 3 mg/mL and 1 mg/mL, respectively.
- F-3 contained xylometazoline HCl only, at a concentration of 1 mg/mL.
- F-4 contained indomethacin only, at a concentration of 3 mg/mL.

Experiment

All the animals underwent anesthesia, provided by intraperitoneal injection of ketamine hydrochloride and xylazine hydrochloride, at a dose of 75 mg/kg and 10 mg/kg, respectively. The method used for inducing rhinosinusitis has been previously described in the literature[10,11]. A standardized solution of platelet activating factor (Sigma Chemical Co., St. Louis, USA) in ethanol was prepared at a concentration of 1 mg/mL. This was then diluted further with normal saline containing 0.25% bovine serum albumin. All animals had induced rhinosinusitis. These rats received a nasal application of 50mL of the platelet-activating factor solution at a concentration of 16 µg/mL. The control animals, i.e., those in which rhinosinusitis was also induced, had 50 µL of normal saline applied, also containing 0.25% bovine serum albumin and ethanol. These animals were designated group A, the negative controls. There were four groups for the experiment, namely:
- Group 1: 7 rats. This group were controls with sinusitis. The only intervention was application of inactive 0.9% saline.
- Group 2: 7 rats. This group also had sinusitis. The intervention was nasal application of xylometazoline and indomethacin in the combined formulation.
- Group 3: 7 rats. This group also had sinusitis. The intervention was nasal application of xylometazoline only.
- Group 4: 7 rats. This group also had sinusitis. The intervention was nasal application of indomethacin only.

Histopathological Examination

The entire group of rats was euthanized on the 21st experimental day. This was achieved by injecting an overdose of ketamine (250 mg/kg) into the peritoneum. The animals' heads were then removed and placed in a fixative solution of formaldehyde 10% overnight, to preserve the microarchitecture of the respiratory and olfactory mucosa. The fixed heads were then decalcified and sectioned in a coronal plane at the midpoint of the nasal cavity, so that both the maxillary sinus and olfactory region were sampled. These thick sections were embedded in paraffin wax and then cut into 5 µm thin sections for haematoxylin and eosin staining prior to histopathological evaluation.

The histological assessment was undertaken with an Olympus BH-2 light microscope (Olympus Optical Company Ltd, Tokyo, Japan). The examiner was a senior histopathologist (DB). Blinding was employed. Each section was evaluated histopathologically, according to a semi-quantitative grading protocol, as follows:

- Level of congestion in the blood vessels: Grade I – vessels were not congested or only minimally so; Grade II – vessels mildly or moderately congested; Grade III – vessels severely congested and red blood cells found external to the vessel.

- Intensity of infiltration by cells of inflammatory type: Grade I – no inflammatory cells present; Grade II – some groups of inflammatory cells within the mucosal or submucosal layers; Grade III – the mucosa (+/- submucosa) contains a severe level of inflammatory infiltrate and the mucosal architecture may potentially be disrupted.

-Extent of injury to epithelium: Grade I – no injury noted; Grade II – intraepithelial degeneration present to some extent; Grade III – the epithelial cells have lost their polarity and may or may not have lost their cilia[10,11].

For each rat, the grading was given on the basis of two sections, one showing nasal respiratory and one olfactory epithelium. Furthermore, scores were obtained for each animal by allocating a numerical value to each grade, with grade I scored 1, grade II scored 2 and grade III scored 3. This applied to each of the graded histological appearances. The scores were then totaled for all the animals in each group. The mean score was then obtained by dividing the total overall score for each group by the number of members of the group[10,11].

Statistical Analysis

The statistical analysis was undertaken using the SPSS 15.0 statistical application (SPSS, Inc., Chicago, IL, USA) running on Windows. The Pearson and Fisher's exact Chi-square test were employed to assess statistical significance. A p-value <0.05 was considered indicative of statistical significance.

Results

For the animals in group 1 (the controls), the mucosa had sustained a significant level of damage and the vessels were highly congested. Inflammatory cells were extensively infiltrating the mucosa. (Figure 1 – A1, 2, 3).

In group 2, by contrast, the vessels were hardly congested and there were very few infiltrates. The epithelium appeared completely intact (Figure 1 – B1, 2, 3). Furthermore, when groups 1 and 2 were compared in terms of congested vessels, inflammatory cellular infiltrates and injury to the epithelium, the differences reached statistical significance, with p-values of <0.01, >0.001 and <0.001, respectively.

Comparison of groups 2 and 4 with the control group also revealed statistically significant dif-

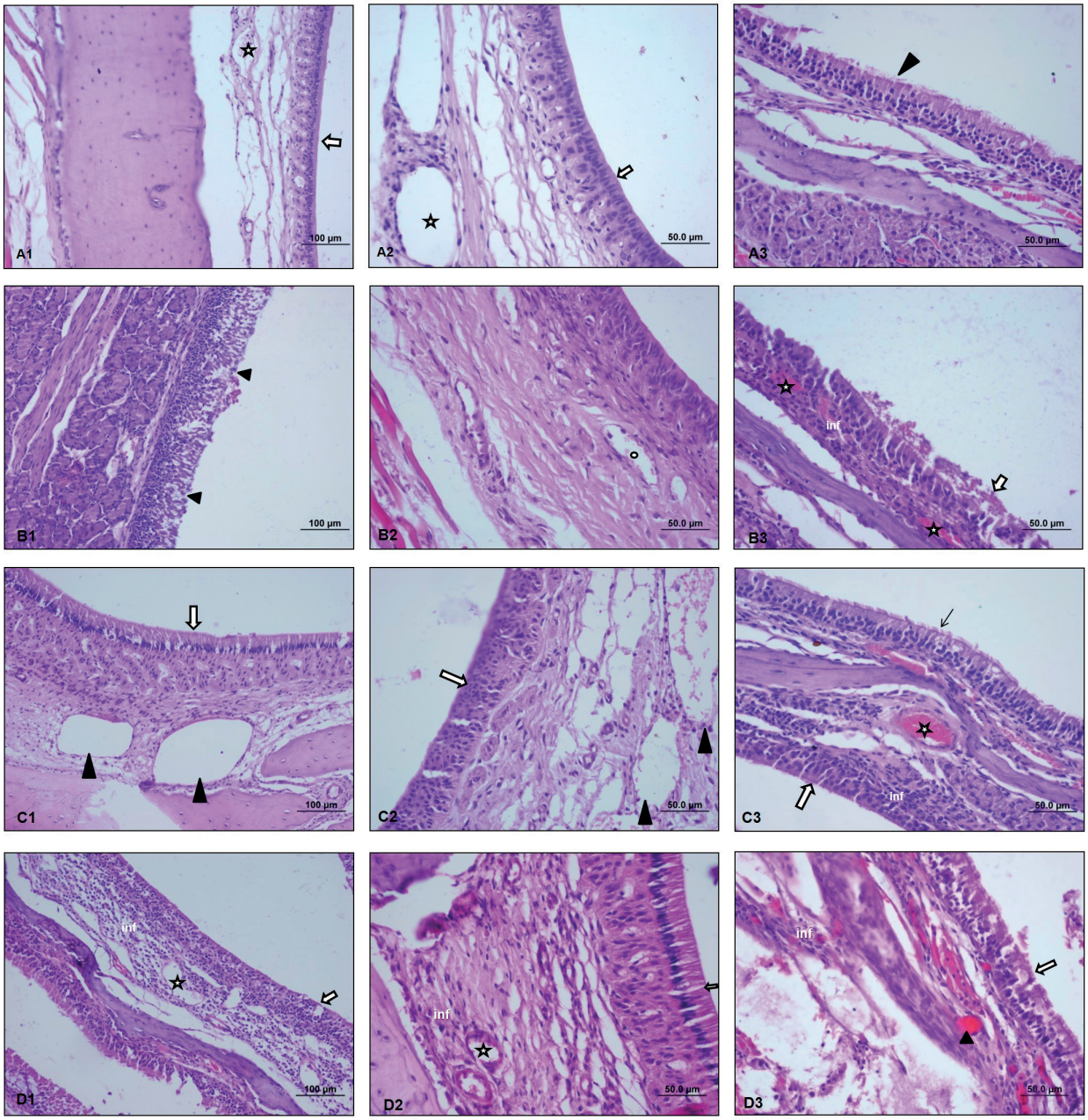

Figure 1. A-D (A1, A2, A3 – B1, B2, B3 – C1, C2, C3 – D1, D2, D3), Light microscopic appearance of haematoxylin and eosin-stained mucosa (respiratory and olfactory) from each group of rats. There were 7 animals in each group.

ferences in terms of cellular infiltrates (p<0.001) and damage to the epithelium (p<0.001). For the degree of congestion of the vessels, however, the difference between groups was not at the level of statistical significance (p<0.071).

Groups 3 and 4 differed at a statistically significant level in terms of degree of congested vessels, cellular infiltrates, and damage to the epithelium (p<0.025 and p<0.001). The sections from rats in groups 2 and 3 had a lower degree of congested vessels, which may be due to the actions of xylometazoline.

Group 1 (A1, A2, A3) – the Controls

Appearance with light microscopy. Normal-appearing olfactory epithelium () and blood vessels () (A1, A2) and normal respiratory epithelium (►) (A3) were noted. (Haematoxylin and Eosin (HE), scale bar: 100 μm, scale bar: 50.0 μm).

Group 2 (B1, B2, B3) – Indomethacin and Xylometazoline Treatment

On light microscopy, the olfactory epithelium was noted to be extensively injured (►) (B1) and the vessels in the lamina propria were dilated () (B2). The respiratory epithelial structure indicated widespread injury, with loss of cilia () and congested vessels (). The lamina propria contained inflammatory infiltrates (inf) (B3) (HE, scale bar: 100 μm, scale bar: 50.0 μm).

Group 3 (C1, C2, C3) – Xylometazoline Only Treatment

Appearance with light microscopy. The olfactory epithelium is injured () (C1, C2, C3) and the vessels within the lamina propria are dilated (►) (C1, C2). The respiratory epithelial appearances indicate widespread injury (→), with congested vessels () in the lamina, which also contains inflammatory infiltrates (inf) (C3) (HE, scale bar: 100 μm, scale bar: 50.0 μm).

Group 4 (D1, D2, D3) – Indomethacin Only Treatment

Light microscopic appearances indicate the olfactory epithelium has sustained injury () (D1). The vessels within the lamina propria are dilated () and there are inflammatory cellular infiltrates (inf) present. The respiratory epithelium is severely injured, and the cilia have been lost (). The vessels within the lamina propria are congested (►) and a cellular inflammatory infiltrate (inf) is present here, too (D3). (HE, scale bar: 100 μm, scale bar: 50.0 μm).

Discussion

Clinical benefit from the use of any medication depends primarily on the efficacy of the agent selected, but a second important factor to consider is how the drug is delivered to the area where it is required and how long it remains *in situ*, so that it can exert its effects. In our study, the aim was to formulate the agent in such a way that it remained in place for a prolonged period. In this, the form chosen proved successful. We chose an anti-inflammatory agent, i.e., indomethacin, and an agent promoting nasal decongestion, namely xylometazoline. It is probable that the success shown with these two model agents can be replicated with other medications, if they can be formulated in a way ensuring they remain *in situ* for longer than usual, allowing the full benefit to be obtained. Our hope is that this study will provide a stimulus for other researchers to consider innovative ways to formulate specific drugs so as to render them thixotropic. Thixotropic behavior is a term applied to how certain non-Newtonian fluids react under specific conditions. Thixotropic materials exhibit a changing viscosity over time. There are multiple fluids of gel or colloid type which exhibit pseudoplasticity or thixotropy. In such materials, a shearing stress, which may be provided by shaking a container of the material, disrupts the solid matrix and the material then acts like a liquid. This can be beneficial when an agent in gel form needs to be applied to an area as a liquid before it then reforms into a gel. The difference between pseudoplasticity and thixotropy is that, in the former, the material only becomes liquid if the shearing force applied grows over time, whereas, in the latter, the liquid state continues to prevail, even if the shearing force applied is constant over time. As thixotropic or pseudoplastic materials come to rest, they resume the matrix configuration characteristic of the gel form. This gel then remains in a stable condition.

It has already been conclusively established that rhinosinusitis is linked to nasal and sinusal colonization by specific bacteria. The presence of these bacteria worsens the degree of inflammation. The inflammatory response, whether acute or chronic, involves production of reactive oxygen species (ROS) by various cell types, including macrophages. These ROS function as important signals which orchestrate the inflammatory response. The high level of synthesis of ROS by polymorphonuclear neutrophils involved in inflammatory responses causes damage to tis-

sues and prevents normal functioning of the endothelium. The movement of cellular and humoral immune defenses depends on their ability to pass into the tissues *via* the endothelial lining of the capillaries. During inflammation, polymorphonuclear neutrophils generate conditions which cause oxidative stress to the endothelium. In response, the junctions between endothelial cells relax, allowing immune effector cells to pass through. Although inflammation has the benefit of eliminating foreign bodies or pathogens, it also causes damage to the tissues. Avoiding this tissue damage is the rationale for using anti-inflammatory agents, whether steroids or NSAIDs[12,13].

The use of topical steroid within the nasal cavity is common in cases of chronic rhinosinusitis, although the details of dosage, dose frequency, formulation, and specific corticosteroid vary widely[13,14]. The volume of corticosteroid that is delivered to the mucosal lining of the paranasal sinuses depends on the way the preparation is formulated. Some common delivery methods include nasal drops, sprays, aerosols, nebulizers, or atomizers. Alternative techniques exist, such as direct injection into the sinuses *via* a cannula or irrigation of the nose with a neti pot or squeeze bottle. These more direct techniques probably allow for a greater amount of agent to contact the mucosa and are especially valuable following surgical operations on the sinuses[13,14].

The mode of action of corticosteroids is at gene transcription level. These agents cause fewer gene transcripts of pro-inflammatory molecules to be synthesized, as well as upgraded transcription of anti-inflammatory mediators. They decrease expression of chemotactic signaling molecules and reduce infiltration of the airways by immune cells[15].

Whereas topically applied nasal corticosteroids are common, it is unusual to use other types of anti-inflammatory agent in this way. One advantage of topical NSAIDs is their ability to strongly inhibit localized inflammation without the potential systemic side effects associated with oral administration.

Conclusions

In the future, topically applied intranasal NSAIDs will be valuable formulations. Innovative types of formulation, such as those demonstrating thixotropic behavior, permit the agent to remain in prolonged contact with the nasal and sinusal lining. Alongside increased efficacy, these preparations will also improve the side effect profile of NSAIDs, largely eliminating systemic effects.

Conflict of Interest

All authors declare that they have no conflict of interest.

Ethics Approval

Ethical approval for the research, including surgical procedures, was granted by decree No. 172 of the Animal Ethics Committee at Eskisehir Osmangazi University.

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Authors' Contributions

Murat Kar: Planning, designing, literature survey, interpretation of the results, active intellectual support.
İskender Ince: Planning, designing, performing the study, interpretation of the results, active intellectual support, writing.
Cafer Yildirim: Planning, designing, performing the study, literature survey, interpretation of the results, active intellectual support.
Dilek Burukoğlu Dönmez: Planning, designing, data collection, performing the study, literature survey, interpretation of the results, active intellectual support, writing.
Yeşim Karasulu: Planning, designing, literature survey, interpretation of the results, active intellectual support.
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