

Review Article

The Inferior Nasal Turbinate of Sheep as a Model for Studying Post-Interventional Upper Airway Mucosal Remodeling

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Abstract

After using the inferior nasal turbinate (INT) of the sheep to study various surgical interventions, radiofrequency tissue volume reduction (RFTVR) appears to be the less invasive technique currently available to reduce the INT non-osseous volume. The histologic parameters of wound healing such as fibrosis, submucosal interstitial volume, epithelial cell lining necrosis, inflammation and submucosal vascularization provide the measures upon which one should rely in order to compare various surgical (or other) interventions. From a molecular perspective, fibronectin, collagen III, CD68 and matrix metalloproteinase-9 (MMP-9) are candidate control parameters and / or modulators of the wound healing and remodeling processes at the inferior nasal turbinate.

ABBREVIATIONS

INT: Inferior Nasal Turbinate; RFTVR: Radiofrequency Tissue Volume Reduction; MMP-9: Matrix Metalloproteinase – 9; MEC: Monopolar Electrocautery; UTR: Ultrasound Tissue Reduction

INTRODUCTION

Inferior nasal turbinate hypertrophy is a common rhinologic problem. Various techniques and different protocols have been applied to solve the problem, with varying degree of success.

Histopathology after interventions at the non-osseous part of the inferior nasal turbinate

Based on recent findings in a sheep animal model, radiofrequency tissue volume reduction (RFTVR) seems in the longer-term (namely 8 weeks after surgery) to be associated with more pronounced fibrosis, less necrosis of the cells comprising the superficial mucosal lining and more extensive reduction of the volume of the interstitial inferior nasal turbinate volume than other techniques, such as monopolar electrocautery (MEC) [1]. Additionally, RFTVR causes less submucosal inflammation (in the form of neutrophilic infiltration) and less vascularization compared to MEC. As a result, an impairment of the microcirculation (possibly caused by the respective extensive fibrosis) at the inferior nasal turbinate may cause the long-lasting effects of RFTVR. This kind of induction of extensive fibrosis has also been

observed in a variety of other tissues and pathologic conditions, such as in auricle keloids [2].

Epithelial cell necrosis and submucosal inflammation at postoperative week 8 in sheep did not differ significantly between RFTVR and control group, a finding that lends support to the minimal invasiveness of the technique. On the contrary, inferior nasal turbinate tissue remodeling is dominated by inflammation and epithelial mucosal lining cellular necrosis in MEC suggesting a much more invasive way of intervention.

The advantage of performing INT surgery using RFTVR is that a lower temperature is required to ablate the tissue than is the case with electrocautery. With RFTVR, the temperature of the target tissue is quite localized and ranges from 60 °C to 90 °C, which limits heat dissipation and damage to adjacent tissues. Temperatures caused by MEC are higher (at the range of 750 to 900 °C) and result in significant heat propagation. As a result, RFTVR is considered to be more accurate, minimally invasive, and to cause less collateral tissue injury.

In another study of sheep tissue specimens of INT mucosa after MEC and ultrasound tissue reduction (UTR) the normally present submucosal loose connective tissue was replaced by extensive fibrosis with scattered subepithelial islands of inflammatory cells in UTR-treated specimens [3]. A marked decrease in large venous sinusoids was observed throughout the submucosal stroma. Although partial and/or complete epithelial denudation

was found in large regions of the mucosa alongside normal-appearing, pseudo-stratified, ciliated columnar respiratory epithelium in 1-week samples, this epithelial denudation was almost absent at the 8th postoperative week. A well-defined basement membrane was present across the treated areas in all UTR-specimens. In MEC-treated INTs a partial and / or complete epithelial denudation was found in larger regions of the mucosa than in UTR-treated specimens. In addition, in some MEC-treated INT specimens the continuity of the basement membrane was focally disrupted.

Molecular perspective

At the molecular level, immunoreactivity for fibronectin, collagen III and matrix metalloproteinase-9 (MMP-9) at the 8th post-operative week was significantly higher than controls, while immunoreactivity for CD68 was higher, although not significantly, compared to controls. Relatively strong statistical correlations have been found between CD68 immunostaining and epithelial cell necrosis in RFTVR-treated INT specimens [4].

MMP-9 concentrations in the extracellular matrix of the paranasal sinus mucosa have been significantly correlated with healing quality and paralleled the concentrations of MMP-9 in nasal secretions in humans [5]. The amounts of MMP-9 in the nasal fluids were significantly and independently predicted by the number of neutrophils and macrophages within the paranasal sinus submucosal tissues. Nonetheless, in the inferior nasal turbinate in sheep, the tissue concentration of MMP-9 did not seem to closely parallel the submucosal tissue concentration of inflammatory cells. Additionally, there is paucity of data on a comparison between surgically treated and untreated mucosa in human studies. From another point of view, a possible explanation of the discrepancy in these results may be that the tissue damage mechanisms is different between surgical trauma due to RFTVR and trauma caused by cold instruments used in sinus surgery.

Results of tissue immunohistological staining should be interpreted with caution. Several immunohistochemical studies have indicated that CD68 antibodies also react with other haematopoietic and non-haematopoietic cell types and that the intensity of CD68 staining in individual cell types depended on the antibody clone and the fixation technique. Care should be taken when distinguishing macrophages from fibroblasts / stromal cells in paraffin sections after formalin fixation since both cell types are stained highly positive for CD68 [6]. It has therefore been suggested that CD68 is not a selective macrophage marker but rather a lysosomal protein that is enriched in macrophages.

In the aforementioned study of sheep tissue specimens of INT mucosa after MEC and ultrasound tissue reduction (UTR) at the end of the study period (week 8 postoperatively) collagen III, fibronectin and MMP9 were increased in both groups compared to levels in the control group [3]. CD68 immunoreactivity was found higher in MEC group but not in UTR group when compared to the control group. After statistical analysis, fibronectin subepithelial immunoreactivity showed a substantial negative correlation with mucosal epithelial cell necrosis, a substantial positive correlation with fibrosis in MEC-treated specimens and a significant positive correlation with sinusoid engorgement and submucosal vascularization in UTR-treated specimens. Collagen

III tissue immunoreactivity showed a particularly significant negative correlation with sinusoid engorgement in MEC-treated specimens.

Fibronectin and collagen III seem to follow in this model system parallel changes in their respective expression patterns in both interventional groups (i.e. MEC- and UTR- treated turbinates), compared to the control group, suggesting a possible major role in wound healing and tissue remodeling, especially in fibrosis formation.

Fibronectin (FN) is a major component of the extracellular matrix and can exist in two main forms: plasma and cellular FN. Cellular fibronectin is able to induce fibroblast differentiation [7]. Fibronectin requires the help of cells to assemble into a functional fibrillar matrix. Cell migration and fibrillogenesis is initiated and governed by cell surface integrins that bind to specific sites in the FN molecule [8]. Apart from its role in the maintenance of the epithelial and extracellular matrix (ECM) integrity, fibronectin also plays an important role in regulating the vascular remodeling response [9]. Supported by findings of the aforementioned studies [3,4], fibronectin may have a major recruiting and remodeling role at both the ECM and vascular level during INT wound healing.

The organization and maintenance of type I and III collagen fibril network in the ECM depends on the presence of an organized, fibrillar fibronectin matrix [10]. Deposition of collagen III into matrix fibrils may be fibronectin-dependent but can also occur through interactions with an ECM component other than fibronectin. The requirement of matrix fibronectin for collagen I and III deposition and maintenance suggest that fibronectin fibrils form a template for collagen I and III deposition [10].

The CD68 is been used as an established marker of tissue macrophages in nasal musosa wound healing studies [11]. Macrophages, which arrive at the wound at a later stage than any other inflammatory cells, clear the wound of all matrix and cell debris including fibrin and spent neutrophils [12]. The fact that macrophages are present in baseline levels at week 8 postoperatively in UTR-treated INTs suggests that the wound healing process is very advanced and rather completed at this stage. On the contrary, macrophages are still present at week 8 in MEC-treated specimens, suggesting that there is still an ongoing, more prolonged, wound healing process compared to UTR-treated INTs [3].

Neutrophils were suggested to be the major source of increased MMP-9 expression, which was linked to poor healing quality in humans [5]. Nonetheless, macrophages may also be an important source of MMP-9. In the study by Nousia et al. [3], MMP-9 concentration was shown to reach its peak at week 8 in MEC-treated INTs, suggesting again (as is the case with CD68 / macrophages) a delayed functional role for neutrophils after this treatment modality and a resulting delayed wound healing process, compared to UTR-treatment (in which MMP-9 concentration peaks at postoperative week 3). Given that macrophages can also be a source of MMP-9 in asthma [13], the aforementioned results should be extended in the future by specific separate analyses on the local MMP-9 release and activation processes as well as recruitment and activation of macrophages and neutrophils.

CONCLUSION

After using the inferior nasal turbinate (INT) of the sheep to study various surgical interventions, radiofrequency tissue volume reduction (RFTVR) appears to be the less invasive technique currently available to reduce the INT non-osseous volume. The histologic parameters of wound healing such as fibrosis, submucosal interstitial volume, epithelial cell lining necrosis, inflammation and submucosal vascularization provide the measures upon which one should rely in order to compare various surgical (or other) interventions. From a molecular perspective, fibronectin, collagen III, CD68 and matrix metalloproteinase-9 (MMP-9) are candidate control parameters and / or modulators of the wound healing and remodeling processes at the inferior nasal turbinate.

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