ORIGINAL CONTRIBUTION

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Ultrasound submucosal inferior nasal turbinate reduction technique: histological study of wound healing in a sheep model*

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SUMMARY

Aim of study: *To describe histological changes after application of an ultrasound tissue reduction (UTR) technique in a newly introduced sheep model to study inferior nasal turbinate mucosal wound healing.*
Materials and Methods: *An experimental study in a sheep model. Stromal fibrosis, submucosal interstitial space volume (ISV), mucosal epithelial cell necrosis, mucosal inflammation and blood vessels' engorgement in inferior turbinate samples were assessed after application of UTR using hematoxylin/eosin staining in 12 turbinate samples after different time points. Histological grading was performed using a 4-point scale*
Results: *After one week, extensive development of fibrosis ($p = 0.006$) and significant reduced stromal ISV ($p < 0.001$) compared to controls were observed. The degree of fibrosis and the stromal ISV remained constant during the 8-week period. The extensive epithelial cell necrosis observed at week 1, diminished later, so that at 8 weeks no significant difference with controls in the necrosis extent was noted. Significant reduction of engorgement of blood vessels in the submucosal layer was noted after 8 weeks ($p = 0.008$, compared with baseline). Mucosal inflammation, while pronounced at week 1 ($p = 0.005$, compared with baseline), was normalized at week 8.*
Conclusion: *Due to the rapid induction of extensive fibrosis, to the limited inflammation reaction, to the moderate degree of epithelial necrosis, to the reduction of subepithelial ISV and the persistence of these features till week 8 at least, UTR emerges as an effective minimally invasive technique for inferior nasal turbinate volume reduction.*

Key words: wound healing, histology, nasal mucosa, inferior turbinate, ultrasound, surgery

INTRODUCTION

Optimal surgical inferior nasal turbinate reduction technique should strike a balance between effective turbinate volume reduction and preservation of nasal function with avoidance of complications (1). Although some preliminary studies on mucosal changes after ultrasound turbinate reduction (UTR) have been reported in the turbinates of human individuals with chronic hypertrophic rhinitis (2), there are only a few reports describing systematically wound healing after surgical reduction of inferior turbinate tissue. Our aim was to explore both epithelial and sub-epithelial tissue changes occurring after ultrasound turbinate reduction (UTR).

Ultrasound tissue reduction of the hyperplastic inferior turbinates, as well as of other kind of hyperplastic or neoplastic mucosa of the upper airway, have been studied by Ferkel'man and Vinitskiš in the 1970's and have demonstrated its efficacy (3). Due to the increased use of UTR in daily practice, more data about its mechanism of action are mandatory. Ultrasound energy causes tissue collagen and protein denaturation resulting in fibrosis and thrombus formation, leading to sealing of the vessels with a diameter less than 0.5 mm. Care has been taken during the procedures to prevent contact of the probe with the underlying bone and therefore prevent osteitis.

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In this study, a sheep model was developed to maximally profit from an adequate monitoring of the different healing phases. Sheep animal models have already been extensively used for both anatomical and physiological purposes [4-6]. The anatomical similarity of the nasal turbinate in sheep with human concha justifies the use of this model for exploration of complex phenomena such as wound healing.

The aim of the study was to explore histomorphologically the early repair phases in the nasal mucosa after UTR. These findings are designed to increase our knowledge of the fine healing process, as well as on the intrinsic principles of action of UTR.

METHODS

Experimental design

The experimental protocol was approved by the Animal Care and Use Committee of the local Veterinary Service since it was in compliance with Directive 86/609/EEC. Sixteen clinically healthy female sheep, weighing 25-35 kg, were used in the study. The animals were randomly assigned to 2 groups. Under general anesthesia, they were either subjected to unilateral submucosal ultrasound tissue reduction of the inferior nasal turbinates (group U, n = 12) or a sham operation (control group, n = 4). The use of a non-longitudinal design was justified by the fact that previous surgical manipulation for biopsy purpose was susceptible to profound modification of the mucosa's histological structures without a direct link with the UTR tissue damages. Four groups of four sheep were established: a control group, representing the preoperative conditions, and three subgroups of group U, representing the post-operative conditions at week 1 (subgroup U1, n = 4), week 3 (subgroup U3, n = 3) and week 8 (subgroup U8, n = 4), respectively.

Anesthesia – post-operative care

After overnight fasting, the animals were endotracheally intubated and anesthetized with xylazine (0.2 mg/kg, i.m.), atropine (0.04 mg/kg, i.m.) and ketamine (10 mg/kg, i.m.). Anaesthesia was maintained using sevoflurane 2% in oxygen. Post-operatively, the animals received 3 ml of long-acting intramuscular terramycin.

Ultrasound

In the twelve sheep of group U, a Crystal Surgical Ultrasound system (Model Lora Don, Diamant Medical Equipment Ltd, Thessaloniki, Greece), consisting of a main unit and a transducer hand piece probe, was used. The main unit is a low frequency ultrasound generator (44 + 4.4 KHz), which is connected to an acoustical transducer and controlled by a footswitch. The electrical ultrasound frequency oscillations produced by the generator, are transformed to mechanical oscillations, by a positive magnetostriction transducer and transferred to a concentrator, a wave-guide operating tool. The acoustical transducer includes crystal magnetic ferrite, which transforms, based on the principal of the piezoelectric phenomenon, the low frequency ultrasound into mechanical oscillations, which are transferred to the wave guide tools. The ultrasound system operates on a three amplitude scale, corresponding to wavelength amplitudes of 20, 30 or 40 μm. For the present study, only the 30 μm - amplitude setting was used. The probe was inserted submucosally in the caudal part of the head of the inferior nasal turbinate and was kept activated during the entire period that remained within the inferior nasal turbinate. The probe was introduced into and removed from the caudal purely submucosal (non-osseous) part of the inferior nasal turbinate with a continuous, straight longitudinal sliding motion beginning at the head, reaching the very end of the tail and finally being

Table 1. Overview of the grades of the morphological changes over time after application of submucosal URT. Histological grading of the changes was performed semi-quantitatively using a 4-point scale (0 = absence, 1 = mild, 2 = medium, 3 = pronounced). The respective p-values resulting from comparisons of the changes between weeks as well as between treated and control groups are also depicted.

Time	Fibrosis	ISV	Necrosis	Inflammation	Blood vessels
Control group					
Median value (width)	0	1	0	0 (0 – 2)	2,50 (2 – 3)
Mean value ± SD	0	1	0	0,60 (0,84)	2,50 (0,53)
1st week					
Median value (width)	3 (2 – 3)	0	0,5 (0 – 2)	3 (2 – 3)	1 (1 – 3)
Mean value ± SD	2,75 (0,50)	0	0,75 (0,96)	2,75 (0,50)	1,50 (1,00)
3rd week					
Median value (width)	2,5 (2 – 3)	0	0	1,5 (1 – 2)	1,5 (1 – 2)
Mean value ± SD	2,50 (0,58)	0	0	1,50 (0,58)	1,50 (0,58)
8th week					
Median value (width)	2,5 (2 – 3)	0	0 (0 – 1)	0,5 (0 – 2)	1,0 (1 – 2)
Mean value ± SD	2,50 (0,58)	0	0,25 (0,50)	0,75 (0,96)	1,25 (0,50)
1st vs 3rd week	0,495	1,000	0,131	0,032	0,739
1st vs 8th week	0,495	1,000	0,405	0,025	0,850
3rd vs 8th week	1,000	1,000	0,317	0,222	0,495
1st week vs control	0,006	<0,001	0,020	0,005	0,058
3rd week vs control	0,007	<0,001	1,000	0,070	0,020
8th week vs control	0,007	<0,001	0,114	0,751	0,008

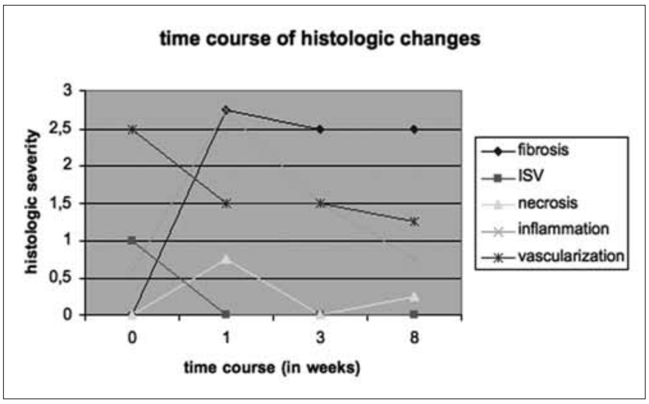

Figure 1. Time course of the mean values of the respective semi-quantitatively assessed severity scores of histologic changes in the control group (0 weeks) and 1, 3 and 8 weeks after application of UTR.

again extracted at the point of introduction at the head of the turbinate, in a direction parallel to the floor of the nasal cavity. The total time needed for each application ranged between 15 and 20 sec (depending on the length of the turbinate). The time needed for the ventro-dorsal motion equalled the time needed for the reverse (dorso-ventral) motion. A part of this period (2-3 seconds) was needed to keep the probe at the end of the procedure at the insertion site to ensure hemostasis. The longitudinal, rapid motion of the probe allows immediate coagulation of the surrounding tissue at its tip.

Morphological studies

The entire inferior turbinate was excised using cold instruments under general anesthesia and tissue samples from the inferior nasal turbinates were prepared for histological examination. Care was taken not to disrupt the delicate mucosal epithelial surface, ensuring the integrity of the samples at the time of retrieval and preparation. Sections were fixed in 10% buffered formaldehyde. The bone of the samples was separated by sharp dissection. The samples were dehydrated with increasing concentrations of ethanol and embedded in paraffin blocks. Serial 3-μm-thick tissue sections were cut at a plane perpendicular to the mucosal surface. Representative sections from the antero-medial aspect of each turbinate were stained with hematoxylin-eosin. The samples were coded at random and analyzed blindly under light microscopy by a senior pathologist (AG). A qualitative assessment of the sections was performed to indicate the type of epithelium, the presence and the intensity of inflammation and fibrosis, and the presence or otherwise population of submucosal glands and blood vessels. Histological grading of the changes was performed semi-quantitatively using a 4-point scale (0 = absence, 1 = mild, 2 = medium, 3 = pronounced) as previously described for a wound healing study [7].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS, Inc., Chicago, IL, USA). Because the distribution of the continuous variables did not appear to be normal, a Shapiro-Wilk test for normality was performed, and data were analyzed using the Mann-Whitney U-test and the Kruskal-Wallis one-way ANOVA, to assess differences between two or more groups of animals, respectively. Post hoc analysis was performed using Mann-Whitney U-test. All tests were two – tailed. Statistical significance was set at the 0.05 level.

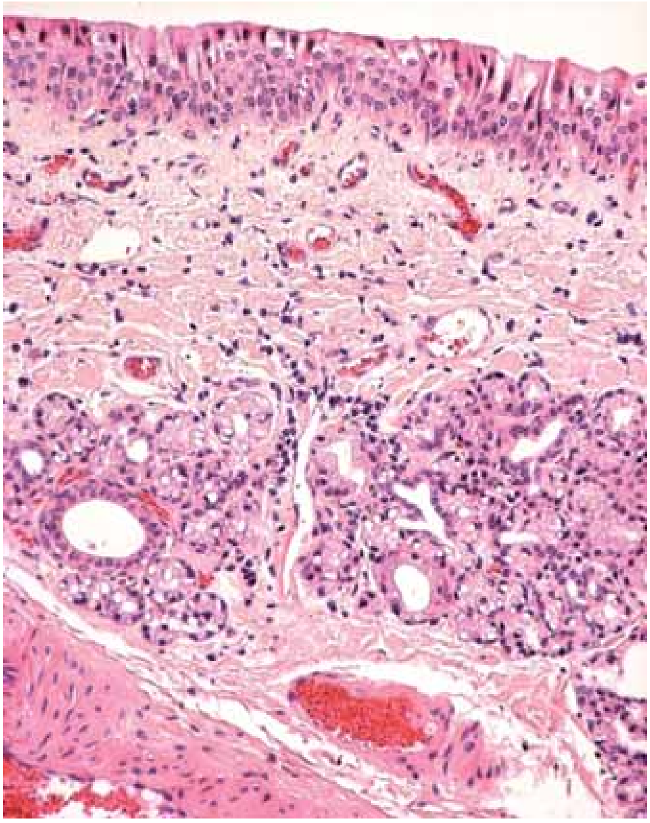

Figure 2. Histological specimen of inferior nasal turbinate mucosa from the control group (hematoxylin – eosin stain, magnification x 20).

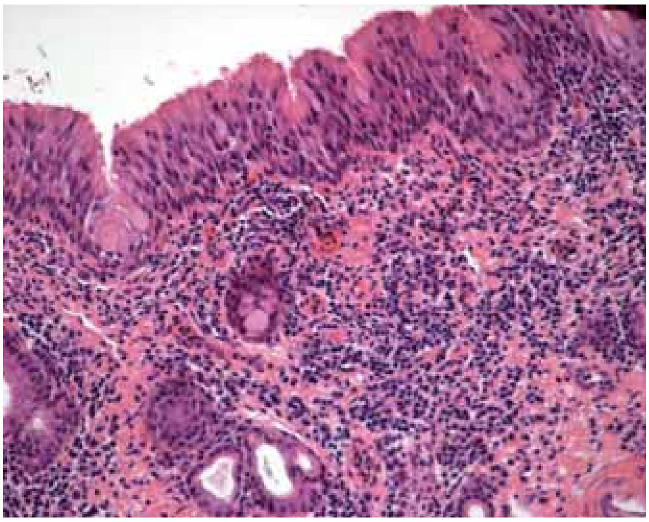

Figure 3. Histological specimen at the 1st week, showing pronounced inflammatory reaction (hematoxylin – eosin stain, magnification x 20).

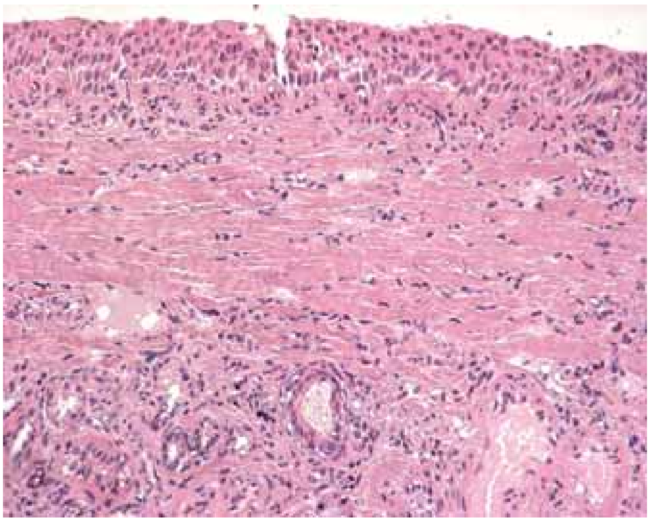

Figure 4. Histological specimen at the 8th postoperative week, showing persisting subepithelial fibrosis after submucosal UTR (hematoxylin – eosin stain, magnification x 20).

RESULTS

The respective p-values resulting after comparison of the semi-quantitative morphological attributes between different time periods, as well as between samples of treated animals and controls are depicted in Table 1 and Figure 1. Histological findings in the control group, at postoperative week 1 and at week 8 are displayed in Figures 2, 3 and 4, respectively.

During the healing process, the general features were the following: the normal loose connective tissue was replaced by extensive fibrosis with scattered subepithelial islands of inflammatory cells. Furthermore, a marked decrease in both submucosal glands and large venous sinusoids was also observed, with apparition of numerous capillaries 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 week 1 samples, this epithelial denudation was almost absent at week 8. Occasionally, the respiratory mucosa was partly or completely replaced by a metaplastic squamous epithelium. Nevertheless, in all postoperative cases, a well-defined basement membrane was present across the UTR-treated areas.

Fibrosis

The technique of UTR induces from week 1 a pronounced fibrosis of the mucosal stroma. No significant differences have been observed in the degree of fibrosis between the 1st and 3rd week ($p = 0{,}495$), between the 1st and 8th week ($p = 0{,}495$) and between the 3rd and 8th week ($p = 1{,}000$) after surgery. The most prominent histological change of all was diffuse fibrosis of the lamina propria extending from the basement membrane superficially to the deeper parts of this layer. The degree of fibrosis was higher in the superficial layers compared to the deeper subepithelial layers. Other significant changes within this layer were a significant reduction in the number of submucosal glands and replacement of the large blood vessels, found in the control group, with small-caliber veins. Although these changes were not yet apparent at week 1, they were prominent at week 8.

Reduction of interstitial space volume (ISV)

In comparison with controls, a significant reduction in the degree of interstitial space volume has been noted throughout the entire study period (1st - 8th weeks, Table 1). The respective area has been replaced by the aforementioned extensive fibrosis.

Epithelial necrosis

In general, during the post-operative period a minimal degree of necrosis of the mucosal epithelial cells with resultant epithelial denudation of the mucosa has been observed (Table 1). The basement membrane was kept intact. The degree of necrosis remained extremely low at the last study points (at the 3rd and 8th postoperative week).

Inflammation

Infiltration of the mucosa with inflammatory cells was quite prominent at the 1st postoperative week but declined progressively during the sub-acute phase, showing a marked and significant decrease ($p = 0{,}025$) between the 1st and 8th postoperative week. At week 8, the observed degree of inflammation was minimal and did not differ from that of the control group ($p = 0{,}751$).

Blood vessel engorgement (vascularization)

The reduction of engorgement of the blood vessels in the submucosal layer noted from the first postoperative week, was progressive and reached statistical significance ($p = 0{,}02$ compared to controls) by the 3rd week, a trend that continued further until the 8th postoperative week.

DISCUSSION

This study demonstrated both the suitability of a sheep model for exploring wound healing and the high integration of remodelling processes involved during the repair process after nasal surgery. The sheep model described in this manuscript can provide the researcher adequate histomorphological information on nasal wound healing and can be proposed as an alternative to the use of human models, which are usually limited for ethical reasons. Also, the need of a large amount of tissue samples for a study like this can be adequately addressed in this model. In humans, to avoid an empty nose syndrome, the resection must be limited and cannot reach the supporting bony or cartilaginous structures. Finally, in the testing phase of new nasal instrumentation, this model can serve as a basis before further studies in human. However, these arguments cannot avoid a final correlation with human findings and a translation of sheep findings to human conditions should be done with caution.

The number of experimental animals was not large. As a result, the statistical power of our results is reduced. Although the number of samples was limited and the design was not purely longitudinal, this study revealed interesting interactions between the different postoperative remodelling parameters. Wound healing, essential to the continuity of human life, involves a continuous sequence of signals and responses in which platelets, fibroblasts, epithelial, endothelial, and immune cells come together outside their usual domains to orchestrate the tissue repair [8,9]. This study revealed a relative stability of tissue remodelling parameters such as fibrosis or oedema while the inflammatory reaction diminishes progressively with time. Interestingly, fibrosis and oedema seem to present different evolution profiles, with relatively high fibrosis scores when oedema is extremely limited. This study provided a first explanation. The reduction in the degree of stromal oedema may be explained by the reduction in local blood flow to the turbinate due to the interstitial and peri-vascular fibrosis with a resultant decrease of engorgement of the blood vessels. This observation was similar to the human repair process demonstrated after sinus surgery [10].

Finally, this study has shown that a postoperative morphological hallmark of URT surgery was the relatively high degree of preservation of the pseudostratified, ciliated, columnar respiratory epithelium and the relative paucity of areas of partial epithelial shedding manifested by a basement membrane covered with a single layer of basal cells and devoid of ciliated, non-ciliated, and goblet cells. In this study, we have shown that the pronounced development of fibrosis together with the reduction of stromal oedema and reduction of engorgement of the blood vessels in the submucosal layer were the key processes deployed by ultrasound leading to turbinate volume reduction. This finding could provide the basis for novel therapies in inferior turbinate enlargement and its subsequent nasal obstruction.

CONCLUSION

Submucosal ultrasound nasal turbinate reduction causes a rapid and sustained pronounced fibrosis and a reduction in oedema of the mucosal stroma with resultant significant tissue volume reduction. Eight weeks postoperatively, inflammation and necrosis of mucosal epithelial cells are minimal. Based on these results, ultrasound tissue reduction of the inferior nasal turbinate has the potential to provide an effective minimally invasive treatment of inferior turbinate enlargement in a clinical setting.

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CONFLICT OF INTEREST

None declared.

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