Rhinology, 44, 259-263, 2006

Submucosal glands and goblet cells in maxillary sinus surgery: an experimental study in rabbits*

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SUMMARY

***Hypothesis**: After sinus surgery poor ciliary activity and disorders in mucus production can lead to an increase in postoperative complications.*

***Purpose of this study**: To evaluate the changes in the ultrastructure of submucosal glands (SG) and goblet cells (GC) after partial or wide surgical removal of maxillary sinus mucosa (MSM).*

***Methods**: Twenty New Zealand White rabbits were divided in two groups of 10 animals. In group A, the mucosa of the right maxillary sinus (MS) was removed. In contrast, in group B only a strip the mucosa around the ostium was removed. After three months the sinus were opened and the mucosa studied by light-, and scanning- and transmision electron microscpy.*

***Results**: After three months in group A all right MS were infected with purulent secretions, and the density of SG with a mean value of 10,4 per mm, standar desviation (SD) 3,36 and GC 81,81 per mm (SD: 3,82). However, in group B the SG were 52,3 per mm (SD: 4,5) and GC 4,45 per mm (SD: 1,23). These results were statistically significant ($p < 0,001$). Histopathological findings showed in group A, after three months, SG with a fewer number of microvilli in the glandular lumen.*

Key words: submucosal glands, goblet cells, maxillary sinus, sinus surgery, rabbits

INTRODUCTION

Secretions from the goblet cells (GC) and the submucosal glands (SG) represent a front-line defense mechanism of the nasal mucosa. The increase in retained mucus in chronic sinusitis is probably the result of poor ciliary activity and disorders in mucus production. An increase in GC density may follow infection, after sinus surgery or exposure to noxious substances [1-3].

To treat some maxillary sinus diseases, surgical procedures are carried out requiring removal of sinus mucosa around the ostium. Postoperatively, ultrastructural and physiological characteristics of the regenerated mucosa are important to facilitate the function of the ostium [4,5].

The purpose of this study was to evaluate the changes in the morphology of SG and GC after minimal or extensive surgical removal of maxillary sinus mucosa (MSM), and also to assess whether the alteration in SG and GC activity increased the risk of sinusal infection.

MATERIALS AND METHODS

Animals

Twenty New Zealand White female rabbits weighing betwen 2,4 and 2,7 kg were used. The animals were anaesthetized with an intramuscular injection of ketamine hydrochloride (70mg/kg). The animals were breathing spontaneously. All procedures carried out on the animals were conducted in compliance with national and local regulations and institutional guidelines for humane use of animals.

Surgery

The rabbits were divided into two groups of 10 animals. In group A, about 1 x 1 cm of the mucosa anterior to the ostium of the right maxillary sinus was completely removed without interfering with the ostium. In the others 10 rabbits, in group B, only a circlular strip of mucosa of 2 mm wide around the ostium was removed (Figure 1).

In all the animals, the areas over the bridge of the nose and maxillary sinuses were shaved and a midline incision was made through the skin and periosteum. The periosteum was lifted over the right maxillary sinus, a window of 5 by 5 mm was opened in the superior-anterior wall using a small hammer and drill (Figure 2).

Animal analysis

The animals were observed for three months. Every two days, the animals were examined for nasal symptoms. After three

Presented in the annual meeting American Academy of Otolaryngology Head & Neck Surgery in New York, September 2004.
*Received for publication: February 27, 2006; accepted: August 17, 2006

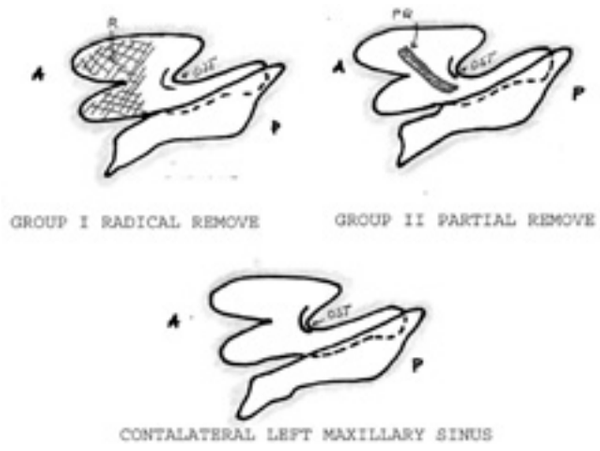

Figure 1. A schematic drawing of the right maxillary sinus cavity seen from the medial aspect. The hatched area shows region of sinus mucosa removed. A: anterior; P: posterior; OST: ostium is indicated by an arrow.

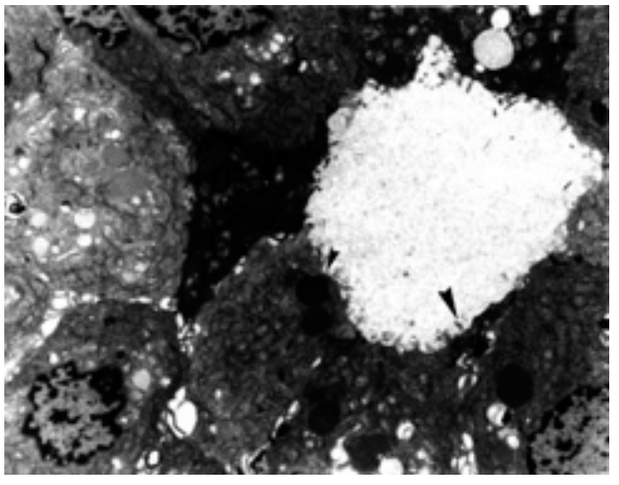

Figure 2. SG in regenerated MSM of group A with transmision microscopy (x 3200). Small arrow: electrodense granules. Big arrow: microvilli on luminal surface.

months the animals were sedated with an intramuscular injection of ketamine hydrochloride (100mg/kg) and tissue fixation was performaded by intra-arterial perfusion. In 5 rabbits from each group the fixation was initiated by perfusion of a solution containing 4% phosphate-buffered formalin. The animals were then painlessly sacrificed. The sinuses were immersed in the same fixative until they were dissected later under a microscope. Five sections were examined from each specimen and the SG and GC counted in 5 fields from each specimen at a magnification of 400x. Using a superimposed graticule showing 1 mm in 100 divisions, it was possible to obtain the number of SG and GC per millimetre of MSM [6,7]. In the other 5 rabbits of each group, fixation was done by perfusion with 2,5% phosphate-buffered glutaraldehyde, followed by procesing for scanning (Hitachi S-2300) and transmision (Zeiss EM 10) electron microscopy. In all animals, specimens of the mucosa were removed from the same sites around the ostium.

After 3 months, the left MS were also opened to assess signs of infection such as pus or hyperaemia of the MSM. Also, the mucosa of these contralateral sinuses was studied using light and electron microscopy. The rabbits were not given prophylactic antibiotics.

Statistics

In the statistical analysis, data were calculated as mean, standard deviation (SD) and range values were compared by the Student-t-test. A value of $p < 0.05$ was considered to indicate statistical significance.

RESULTS

In the three months of the study, the animals were observed to be in good health and with no obvious nasal symptoms such as rhinorrea, sneezing or periorbital swelling. After three months, all the right maxillary sinuses of animals in group A contained purulent secretions. The MSM of group A animals showed a mean value of 10,40 SG per mm, (SD: 3,36), and GC 81,18 per mm (SD: 3,82). However, in group B the mean value of SG was 52,30 per mm (SD: 4,50) and 4,45 GC per mm (SD: 1,23). These different results between groups A and B were statistically significant ($p < 0.001$).

In group A, MSM showed a dense infiltration by plasma cells, lymphocytes and granulocytes. SG had fewer secretory granules, which were more electro-dense, showing variations in size, with a predominance of small granules (Figure 3). In SG secretory cells had fewer microvilli on their luminal surfaces and there was diminished activity of the cell organelles with fewer free ribosomes, smaller mitochondria and Golgi apparatus. The glandular lumina was irregular and contained some reticulated and membranous electro-dense material (Figure 4).

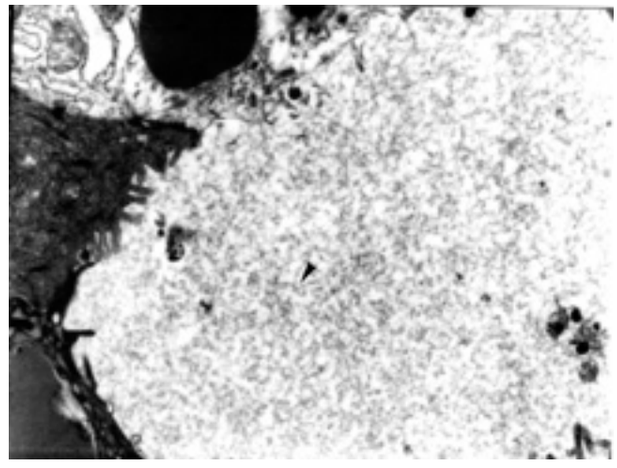

Figure 3. Absence of microvilli in GC of group A. Arrow shows the glandular lumina containing electro-dense material (x 36100).

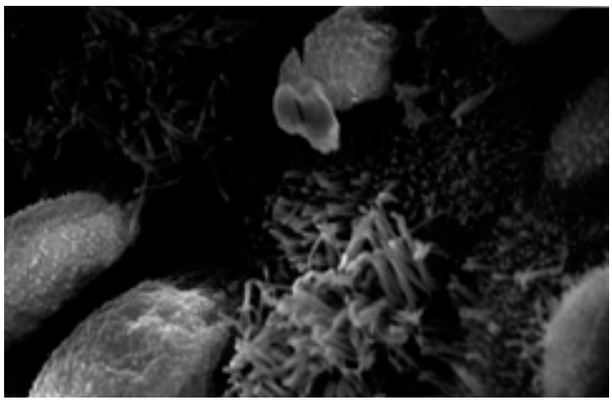

Figure 4. Increase of GC (white surface) in MSM of group A with scanning electron microscopy.

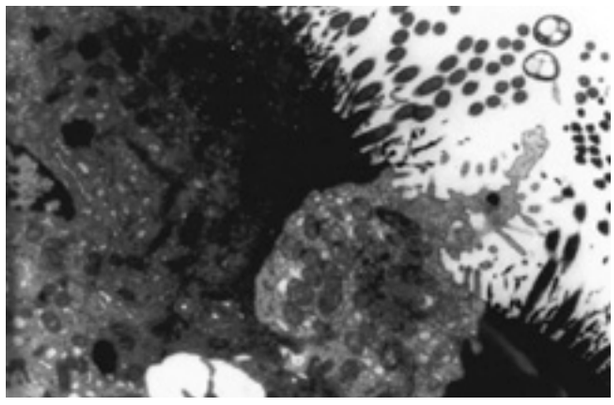

Figure 5. GC in MSM of group A with transmision electron microscopy (x15000).

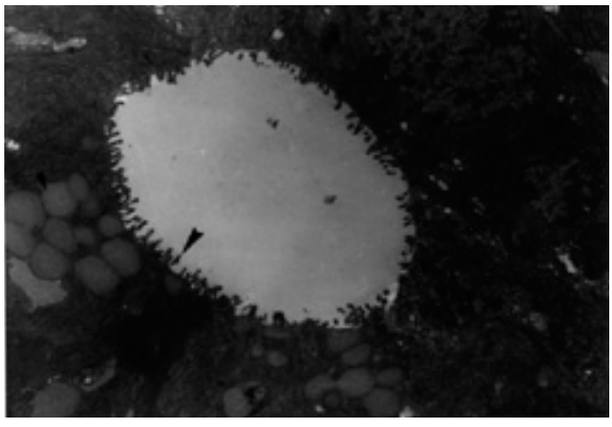

Figure 6. Normal SG in the group B with transmision electron microscopy. Arrow shows many microvilli on luminal surface (x 3400).

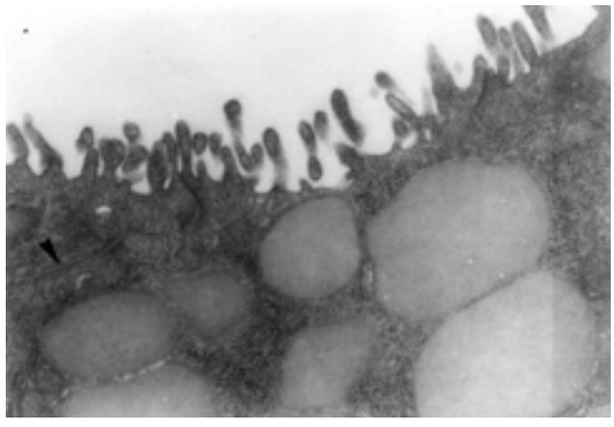

Figure 7. Microvilli on the tubular surface of the SG in group B. Arrow shows intercellular canaliculis (x 30900).

However, GC showed an increased density (Figure 5) and also an increased activity with many secretory granules, ribosomes and mitochondria (Figure 6).

MSM in group B presented SG with many microvilli on their luminal surface and abundant granules which were pale and usually located in the apical portion of the cytoplasm (Figure 7). The secretory cells contain a lot of free ribosomes and mitochondria. The lumen of SG was round or oval in shape. The intercellular canaliculi were dilated near the base of the cells, with finger-like cytoplasmic processes extending between them. However, GC were very scarce and showed little activity.

In the contralateral left sinus of all animals, there were no signs of infection and study by light and electron microscopy of the mucosa showed appearance similar to those found in the right sinuses of animals in group B.

DISCUSSION

The rabbits' maxillary sinus is considered to be a good experimental model for regenerative studies following sinus surgery [8-11]. This epithelial regeneration after surgical removal is considered to be largely complete by one month postoperatively. After selective removal, at 10 and 14-day intervals, re-epithelialisation with ciliated epithelium is observed [12]. In the lamina propia, fibrosis, together with a rich microcirculation and local angiogenesis appear [13]. Reinnervation of mucosa is seen between 4-8 weeks [14]. Changes in the bone of the maxillary sinus such as osteomyelitis have been reported after experimentally induced sinusitis [15].

While GC were normally very scarce in group B who underwent minimal surgical removal of the mucosa, such cells were frequent in group A awho underwent much wider removal. The process of GC differentiation seems to follow a sequential path where intermediate serous secretory cells start to produce an increasing amount of mucous granules [1]. However, in group A, a decrease in density of SG was observed and these SG had only a few granules and mitochondria. The changes in SG might be caused by an alteration in the synthesis of zymogene granules caused by energy depletion and low oxygen tension in the inflamed tissue. This increased secretory rate might also account for the granule depletion in experimental sinusitis in rabbits, with an increase in activity of the energy-requiring enzyme regulating sodium and potassium transport in SG [16,17].

The tubuloalveolar gland system produces considerable

amounts of mucoprotein and acid mucopolysaccharide. However, the mucus of GC differs from that produced by the tubuloalveolar glands in that it is sulphated [18]. Glandular secretions and the quality of the mucus are important aspects of the mucociliary transport mechanism. In MSM of the rabbit, the regenerated mucosa after wide surgical removal had a marked decrease in the number of SG and an increased density of GC, which might be expected to result in increased mucus viscosity and a decreased rate of clearance [19-22].

In group A, with selective removal of mucosa around the ostium, the results of density of GC and SG were similar to the normal MSM in rabbits as has been reported [7]. Although, it is possible, as in patients, that the mere fact of entering the maxillary sinus by an external approach (as seen with dental procedures, or trauma, etc.) may by itself cause secondary infection. An absence of infection after three months in rabbits in whom the maxillary sinus was exposed with the same approach as in the present study, but no procedure was performed on the MSM has been reported [3]. In middle meatal antrostomy, not only may the maxillary sinus mucosa be removed, but by definition it involves ostial enlargement. A widened ostial opening and stripping of MSM could cause changes in SG and GC by increasing the surface area of mucosa that must be regenerated. Further studies are necessary to compare the results in rabbits undergoing ostial openings and stripping of MSM with others cases that only have ostial opening. Partial or selective removal of the periostial mucosa may allow regeneration of more normal mucosa quickly, with improved mucociliary transport and sinus function, without developing infection [23-25]. In clinical studies of chronic maxillary sinusitis pre-operatively and post-operatively to functional endoscopic sinus surgery and Caldwell-Luc operation, the SG were significantly reduced by the Caldwell-Luc operation but not after functional endoscopic sinus surgery [26]. However, a large middle meatal antrostomy with extensive removal of maxillary sinus mucosa could have some adverse effects on the mucosal regeneration and should be reserved for patients who have failed other approaches.

Significant care must be taken in extrapolating these findings in an animal model to disease in patients. The absence of infection in group B and contralateral maxillary sinuses is surprising, since in rabbits a certain incidence of spontaneous sinusitis is anticipated. It is possible that some infection occurred during the period of observation but resolved spontaneously and was not present at the time of re-examination. *Pasteruella multocida*, in particular, has been reported as a major airway pathogen, carried by more than 90 % of a rabbit population studied in the United States. In the majority of cases, the infection is subclinical but when there is interference with the sinus mucociliary transport (for example: radical surgery), the infection may progress [27,28].

In conclusion, there was a marked decrease in the number of SG and an increased density of GC after wide mucosal removal of MSM in rabbits, which might be expected to result in increased mucus viscosity and decreased mucus production. However, partial or selective removal of mucosa in the vincinity of the ostium may allow regeneration of more normal SG and GC without disruption in mucus production.

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