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Submucosal glands after maxillary sinus surgery. An experimental study in rabbits

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Abstract
Thirty New Zealand White rabbits underwent unilateral partial or complete removal of maxillary sinus mucosa in order to evaluate submucosal maxillary sinus glands. After three months, specimens were taken for examination from all operated on and control sinuses. Bacteriological cultures, light and electron microscopy were performed. Histopathological findings showed a decrease in the number of serous glands and significant inflammation was present in the sinus in which there was complete surgical removal. Electron microscopy revealed changes in the secretory cells of the serous glands in the regenerated post-surgical mucosa.

Key words: Maxillary sinus, surgery; Mucous membrane; Rabbits

Introduction

Chronic inflammatory diseases of the nose and paranasal sinuses can inhibit mucociliary transport, which will produce static mucus and a secondary sinusitis. The increase in retained mucus in chronic sinusitis is probably the result of both poor ciliary activity and increased mucus production (Tos and Mogensen, 1984).

Surgical removal of the sinus mucosa for such disorders raises questions concerning mucosal regeneration and subsequent submucosal gland function. Certain surgical procedures carried out on the paranasal sinuses include partial or total removal of the mucosal lining (Stammberger, 1986).

The purpose of this study was to evaluate the changes in the ultrastructure of the submucosal glands after complete or partial surgical removal of maxillary sinus mucosa.

Materials and methods

Thirty New Zealand White female rabbits weighing between 2.5 and 2.8 kg were used. The animals were anaesthetized with an intramuscular injection of ketamine hydrochloride (70 mg/kg). The animals breathed 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 in research.

The rabbits were divided into three groups of ten animals. In Group A the mucosa of the anterior cavity of the right maxillary sinus (MS) was completely removed without interfering with the ostium. Group B consisted of 10 rabbits in which only a strip of mucosa measuring 5 by 2 mm was removed between the ostium and the floor of the right MS. In Group C a small opening was made through the anterior wall of the right MS, and the sinus mucosa beneath the drilled hole was cut to gain entry to the maxillary antrum.

In all animals, the areas over the bridge of the nose and maxillary sinuses were shaved and a midline incision was made through the skin and periostium. The periostium was lifted over the right MS, a window of 5 by 5 mm was opened on the superior-anterior right MS wall using a small hammer and drill and the mucosa was identified and removed as described in the above paragraph.

The animals were observed for three months. Every two days, the animals were examined for nasal symptoms. After three months the animals were sedated with an intramuscular injection of ketamine hydrochloride (100 mg/kg) and tissue fixation was performed by intra-arterial perfusion. In five rabbits from each group the fixation was initiated by perfusion of a solution containing phosphate-buffered formalin four per cent. The animals were then painlessly sacrificed. The sinuses were immersed in the same fixative until they were dissected later under a microscope. The mucosa linings were prepared for light microscopy with haematoxylin and eosin, and Alcian blue-PAS (pH

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1.0 and 2.5) stains after immersion fixation in phosphate-buffered formalin four per cent, decalcification in formic acid, paraffin embedding, and sectioning of the whole-mounted nose complex at 5 μm thickness.

Five sections were examined from each specimen and the submucosal glands and goblet cells counted in five fields from each specimen at a magnification of ×400. Using a superimposed graticule showing 1 mm in 100 divisions (Lund, 1988), it was possible to obtain a figure for the number of submucosal glands and goblet cells per millimetre of epithelium by taking the average of the counts for each specimen.

Five rabbits from each group were submitted to a perfusion of a solution containing 2.5 per cent glutaraldehyde in a phosphate-buffered solution. Strips of the mucosa were removed from the same site in all animals. This site was the whole of the mucosa of the anterior cavity of the MS. Special care was taken in order not to include the mucosa removed beneath the drill hole when evaluating the regenerated mucosa.

An area of mucosa 1 × 1 cm was removed from the anterior cavity of the maxillary sinus in group A rabbits, without interfering with the ostium. In the schematic drawing (Figure 1), the hatched area shows the region of the sinus mucosa removed in the present investigation. The sections for examination of Group B were taken from the area where the 5 by 2 mm strip was removed and the nearby areas. Special care was taken in order to avoid a change in the orientation of the mucosa.

After rinsing in phosphate buffer, the specimens were fixed in one per cent osmium tetroxide, dehydrated in a graded series of acetone, and embedded in a mixture of Epoxyresin, Hardener 964, and plasticizer. Ultrathin sections about 70 nm thick were cut with a diamond knife, mounted on square mesh copper 3.05 mm, and contrasted with lead citrate. Ten preparations from each animal were examined in a Zeiss EM 10A transmission electron microscope. Bacteriological cultures from the right MS cavities were taken with cotton bud Reditubs with a AIMES media. The media used for cultivation were blood agar, McConkey agar and haematin agar. After three months, the left maxillary sinuses were opened, in search of signs of infection such as pus or

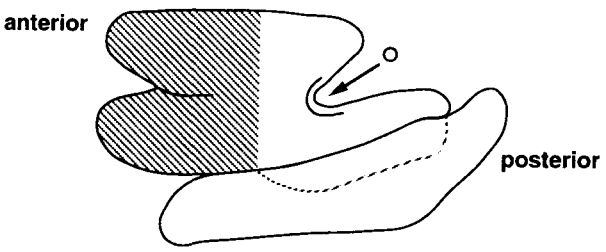

FIG. 1

A schematic drawing of the right maxillary sinus cavity seen from the medial aspect. The hatched area shows region of sinus mucosa removed; ostium—O is indicated by an arrow. (From Forsgren *et al.* (1993) *Annals of Otology, Rhinology and Laryngology* **102:** 459–466 with kind permission of the author and editor.)

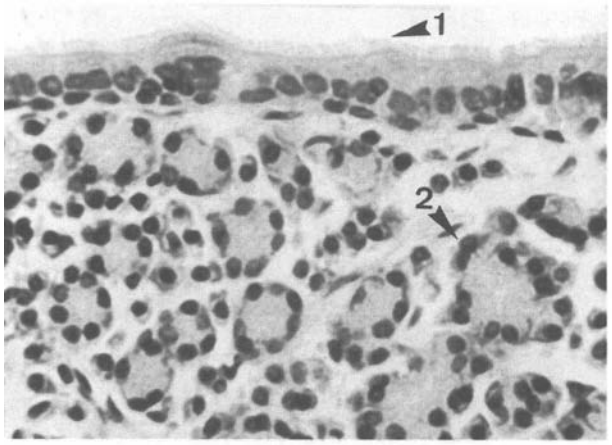

FIG. 2

Normal mucosa of the rabbit maxillary sinus. (1) Ciliated epithelium. (2) Normal serous glands. (H & E; × 450).

hyperaemia of the MS mucosa. Also, the mucosa of the contralateral sinuses was studied by light and electron microscopy.

The rabbits were not given prophylactic antibiotics.

Results

The lamina propria of the rabbit MS mucosa contains numerous serous glands and vessels (Figure 2). The normal submucosal maxillary sinus glands (SMSG) of rabbit are typical serous glands, with pyramid-shaped secretory cells. The mean of SMSG in the normal rabbit MS was 54.5 glands per mm of epithelium, with a standard deviation (SD) 3.20.

There was collagenous connective tissue surrounding the glands with some plasma cells. The glands contained abundant granules which were pale and usually located in the apical portion of the cytoplasm. Their size varied (Figure 3). These abundant granules which were less dense stained magenta with Alcian Blue-PAS technique. However, in the normal

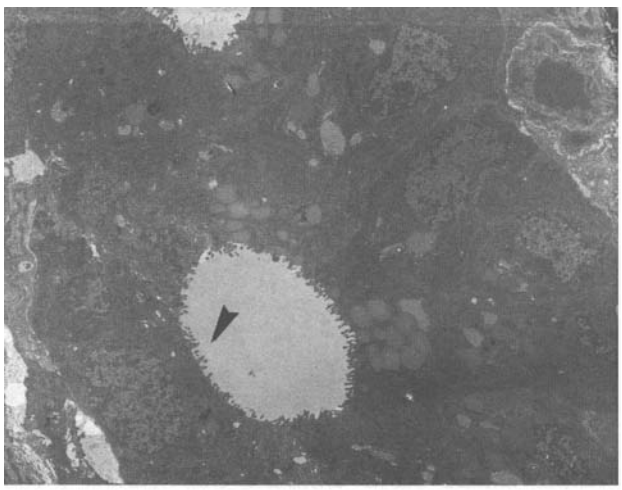

FIG. 3

Normal serous gland of the maxillary sinus by transmission electron microscope. (▶) Luminal surface (× 3200).

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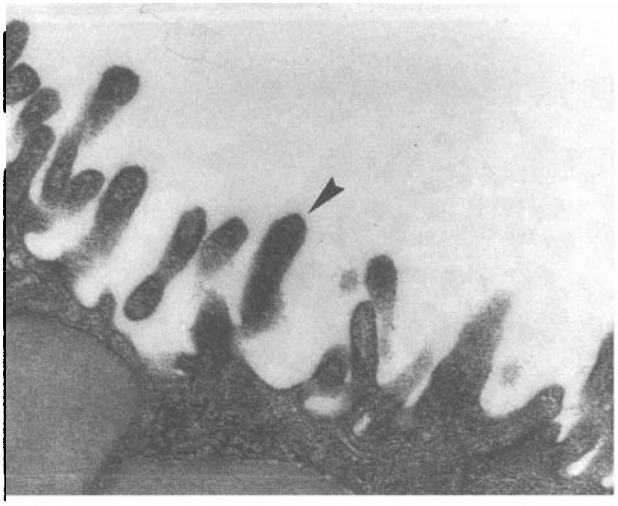

FIG. 4
Microvilli on the luminal surface in normal serous gland of the rabbit maxillary sinus. (× 25 200).

rabbit MS the goblet cells of the epithelium stained blue with this technique. The mean value for goblet cells in the normal MS, in our study, was 2.45 goblet cells per mm of epithelium (SD:0.65).

Normal rabbit serous glands have many microvilli on their luminal surfaces (Figure 4). The lumen of the serous glands is a rounded or oval space. The intercellular canaliculis were dilated near the bases of the cells, with finger-like cytoplasmic processes extending between them. The caniculi tapered off and became narrow as they approached the lumen, showing more junctional complexes in the form of desmosomes.

In normal rabbit SMSG, the secretory cells showed increased activity with many free ribosomes, mitochondria and Goigi apparatus and dilated cisternae of the granular endoplasmic reticulum.

The initial bacteriological cultures from the right MS in 24 animals grew *Pasteurella multocida*. Six cultures showed no microbial growth.

After three months, all the right MS of Group A were infected with purulent secretions. The rabbit MS mucosa of the animals in Group A showed that

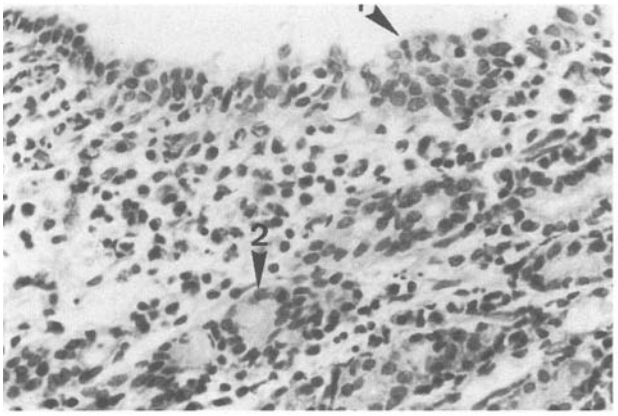

FIG. 5
Maxillary sinus mucosa of one rabbit of Group A after 3 months. (1) Alteration of the ciliated epithelium. (2) Decrease in number of serous glands (H & E; × 450).

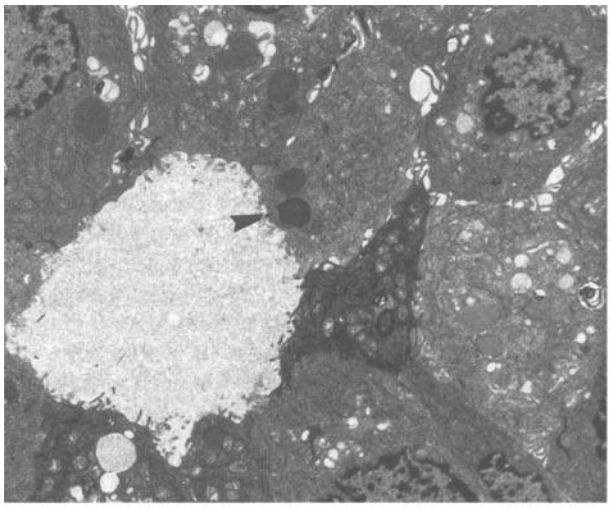

FIG. 6
Serous gland in the regenerated rabbit maxillary sinus mucosa. (▶) Secretory granules (× 3 200).

the right MS mucosa had a decrease in the number of serous glands, with a mean value 14.2 glands per mm (SD:1.36). However, in Group A the mean value for goblet cells was 73.98 goblet cells per mm (SD:3.72).

There was dense infiltration by plasma cells, lymphocytes and granulocytes (Figure 5). In these SMSG there were fewer secretory granules, which were more electro-dense, showing variations in size, with a predominance of small granules (Figure 6).

The secretory cells of the MS mucosa in Group A, had fewer microvilli on their luminal surfaces (Figure 7) and there was diminished activity of the cell organelles with fewer free ribosomes, smaller mitochondria and Golgi apparatus and fewer dilated cisternae of the granular endoplasmic reticulum. The glandular lumina was irregular and contained some reticulated and membranous electro-dense material.

In the other Groups (B and C), there were no important changes in the secretory cells of the serous glands after three months. In Group B the mean

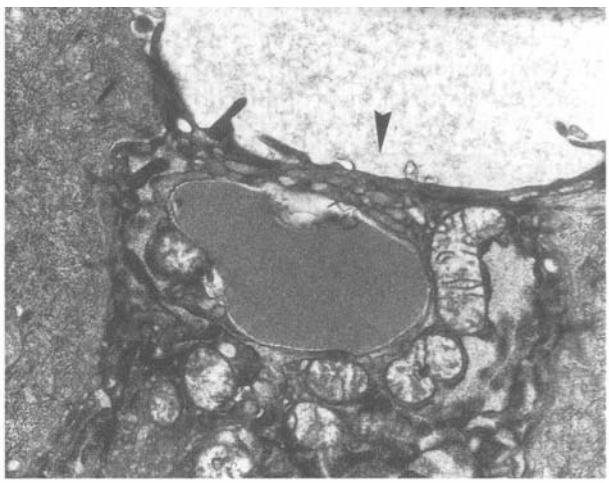

FIG. 7
Absence of microvilli on the luminal surface of the secretory cells of the regenerated sinus mucosa (Group A). (× 36 100)

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value for SMSG was 47.8 per mm (SD:4.2) and in Group C this mean value was of 59.1 per mm (SD:5.1). In Group B the mean value for goblet cells was 4.1 per mm (SD:1.2) and in Group C this mean value was 3.1 per mm (SD:1.1).

After three months, Pasteurella multocida was found in all animals in Group A. In three animals *Pasteurella multocida* was found together with *Flavobacterium*, *Bordetella* and *Moraxella* species. In contrast in Groups B and C, only *Pasteurella multocida* was found.

In the contralateral sinus of all the animals, after three months, there were no signs of infection and a study by light and electron microscopy of the sinus mucosa showed it to be similar to that of the right sinuses in Groups B and C.

Discussion

The tubuloalveolar gland system produces considerable amounts of mucoprotein and acid mucopolysaccharide. The mucus of the goblet cells differs from that produced by the tubuloalveolar glands in that it is sulphated (Taylor, 1992). Glandular secretions and the quality of the mucus are important aspects of the mucociliary transport mechanism. In the rabbit, the regenerated MS mucosa in the operated sinuses had a marked decrease in the number of glands and an increased density of goblet cells, which might be expected to result in increased mucus viscosity and a decreased rate of clearance (Benninger *et al.*, 1989; 1991).

In the rabbit MS mucosal regeneration, the SMSG are decreased in number as described by others (Benninger *et al.*, 1989; 1991). However the regeneration of the SMSG in the rabbit has been questioned (Forsgren *et al.*, 1993).

In the regenerated mucosa of Group A, the SMSG had a few granules and a large number of plasma cells, which play an important role in secretory activity, are seen around these glands. This has also been reported by Westrin *et al.* (1993) in experimental maxillary sinusitis by *Bacteroides fragilis* in rabbits. The changes in the SMSG in the regenerated mucosa of Group A, might be caused by an alteration in the synthesis of zymogen granules caused by energy depletion and low oxygen tension in the inflamed tissue. However, the increased secretory rate might also account for the granule depletion in experimental sinusitis in rabbits (Westrin *et al.*, 1993). It has been demonstrated that the activity of the energy-requiring enzyme regulating sodium potassium transport increases significantly in the rabbit SMSG during purulent sinusitis, indicating an increased secretory rate (Johansson *et al.*, 1988; Miyaguchi *et al.*, 1990).

Normal human MS has very few glands, and most of these are located around the ostium as reported by Tos (1982) and Lund (1988). In normal humans the majority of the SMSG are seromucinous. These glands are mixed comprising mucus-secreting cells and two types of serous secretory acini-basophilic and eosinophilic granules (Friedmann and Osborn, 1982). The zymogen granules of serous cells are fewer in number and paler in density, though variations in their densities and sizes exist (Toppozada and Talaat, 1980). In an attempt to compare this increase in secretion which has been described in human studies, it would appear that the adaptation of the secretory mucosa in each species is limited by its genetically determined transformation potential (Ohashi and Nakai, 1983).

Tos and Mogensen (1984) found that the goblet cell density in human chronic sinusitis was significantly lower than in the normal mucosa, whereas the gland density was six times that of the normal MS. The examination of the biopsy from the normal human MS has shown a range of goblet cell numbers of 35–70/mm (Lund, 1988). However, the dissimilarities observed in the results of different studies can partly be explained by the location of the biopsy, but there may also be local differences in infection defence, epithelial damage after previous infections, impaired blood supply, thickened basement membrane with impaired availability of immunoglobulin or decreased secretion of secretory IgA (Petruson, 1994).

In our study, there are no changes in the SMSG in the group with partial mucosal removal. Partial or selective removal of mucosa may allow regeneration of more normal mocosa, with improved mucociliary transport and sinus function, as described by others (Kennedy and Shaalan, 1989; Ohashi *et al.*, 1991). Forsgren *et al.* (1995) have studied the histopathological mucosal changes occurring in chronic maxillary sinusitis patients both pre-operatively and post-operatively to functional endoscopic sinus surgery and the Caldwell-Luc operation, and they report that SMSG were significantly reduced by the Caldwell-Luc operation but not after functional endoscopic sinus surgery.

The absence of infection in Groups B and C and in the contralateral sinuses is surprising, since in rabbits, a certain incidence of spontaneous sinusitis has to be expected. It is possible that some infections occurred during the time of observation but resolved spontaneously and were not present at re-examination, as reported by Perko and Karin (1992). Spontaneous sinusitis can occur in rabbits with respiratory tract infection, as reported by Kelemen (1955). *Pasteurella multocida*, in particular, has been reported as a major airway pathogen, carried by more than 90 per cent of one examined rabbit population in the United States. In the majority of cases, the infection is subclinical but when there is an interference with the sinus mucociliary transport (for example: radical surgery), the infection can progress (Friedman and Toriumi, 1989).

In conclusion, the SMSG regeneration after radical surgery in the rabbit has two important points: firstly, there is a decrease in the number of serous glands, and secondly these glands appear altered with a fewer number of secretory granules and a fewer number of microvilli in the glandular lumen.

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