

## The effect of ostial opening on experimental maxillary sinusitis in rabbits\*

Yang-Gi Min, Yool-Mo Lee, Bong-Jae Lee, Ha-Won Jung, Sun-0 Chang

Department of ORL, Seoul National University College of Medicine, Seoul, Korea

### SUMMARY

*Obstruction of the natural ostium is known to be one of the major factors in the pathogenesis of maxillary sinusitis. To explore the therapeutic effect of ostial patency, sinusitis was induced in 32 rabbits by obstruction of the natural ostium and inoculation of *Streptococcus pneumoniae*. They were divided into two groups: the first ("open") group included 16 rabbits with experimentally induced sinusitis, from which bone chips for obstruction of the natural ostium were removed after a period of 5 days, and the second ("closed") group included 16 rabbits with continuous obstruction of the natural ostium. The induction of sinusitis was found to be successful in all cases, when examined after five days. The amount of nasal and sinus secretion as well as histopathological findings of the sinus mucosa were investigated with light microscopy, scanning and transmission electron microscopy, two and four weeks after induction of sinusitis. The findings were significantly improved in the "open" group.*

*Key words: experimental sinusitis, maxillary sinuses, ostial obstruction*

### INTRODUCTION

Several factors such as obstruction of the natural ostium, hypomotility of cilia, abnormality of gaseous exchange and abnormality of blood circulation have been implicated in the pathogenesis of maxillary sinusitis. Among them, obstruction of the natural ostium has been regarded as the most important factor (Johansson et al., 1988). The natural ostium is a passageway through which mucociliary transport of secretion and the exchange of gasses between the nasal and maxillary sinus cavities occur (Ballenger, 1985). Since Hilding (1941) reported the effects of operative windows made near the natural ostium of normal sinuses in an animal models, numerous publications on the effects of an opened ostium in experimentally-induced maxillary sinusitis have followed (Reimer and Toremalm, 1978; Kumlien and Schiratzki, 1985). However, few histopathological investigations of these effects have been reported. The aim of the present study was, therefore, to assess the different findings between an "open" and "closed" group by means of light microscopy, scanning and transmission electron microscopy.

### MATERIAL AND METHODS

Thirty-two rabbits, weighing between 1.8 and 2.9 kg, without nasal infection were used. General anaesthesia was induced by intramuscular injection of 50 mg/kg

ketamine, and the skin was prepared. Skin and periosteal incisions were made in the cheek, the anterior maxillary bone was drilled out to a size of 4 X 8 mm, followed by incision of the antral mucous membranes. Sinusitis was induced by blocking the natural ostium with autologous bone chips and by introducing 0.5 ml of a suspension of *Streptococcus pneumoniae* ( $10^9$  cells/ml) into the maxillary sinus cavity. The strains of *Streptococcus pneumoniae* were cultured in a brain-heart infusion, and were then injected into the peritoneum of albino mice. The heart blood was cultured on blood-agar plates and the Pneumococci were isolated.

On the fifth day after, rabbits with experimental sinusitis were divided into four groups (Table 1). Physical findings were noted and autologous bone chips used as blockers were removed in 16 rabbits ("open" group) by the same approach.

Table 1. Experimental groups.

group	N	ostial manipulation after induction of sinusitis	biopsy date
I	8	closed	day 19
II	8	closed	day 33
III	8	opened on day 5	day 19
IV	8	opened on day 5	day 33

After two weeks, eight rabbits from both the "open" and "closed" groups were anaesthetised. The physical findings were checked, and mucosal samples were taken from the medial wall of the maxillary sinus. Physical examination included the collection of nasal secretion and pus from the maxillary sinus.

The mucosal samples were washed in saline, fixed in 10% formalin, embedded in paraffin, stained with hematoxylin and eosin, and examined with a light microscope.

For scanning electron microscopic examination, the samples were washed in saline and fixed by immersion in 2.5% glutaraldehyde for 2 h, dehydrated in a graded series of ethanol, critical-point-dried with liquid carbon dioxide, coated with palladium and gold, and examined in a JSM-820 scanning electron microscope. For transmission electron microscopy, tissues were dehydrated in a graded series in ethanol and propylene oxide, embedded in Epon, ultrathin sectioned with an ultramicrotome, and examined in a JEM-1200 EXII transmission electron microscope.

The remaining eight rabbits from both the "open" and "closed" groups were treated in the same way, four weeks later.

## RESULTS

On the fifth day after the induction of sinusitis, mucopurulent discharges were observed in the nasal cavities of all 32 rabbits. The contents of the maxillary sinus were yellowish or milky-white of colour, and moderate or severe in amount in 15 out of 16 rabbits from the "open" group. The mucous membranes were oedematous and hyperaemic. The patency of the natural ostium was secured after the removal of the bone chips.

Two weeks later, the amounts of nasal and sinus secretions had obviously decreased more in the "open" group than in the "closed" group, but statistical significances were not revealed between the two groups. Light microscopically, inflammatory cells (lymphocytes, neutrophils) were found to be infiltrated more in the "closed" group than in the "open" group, but there was no statistical significance. Upon scanning electron-microscopical examination, a decrease in the number of ciliated cells as well as a loss of stiffness, disturbed orientation of the cilia, compound cilia, and cytoplasmic protrusions were observed in both groups, slightly more in the "closed" group (Figure 1). With transmission electron microscopy, compound cilia, ruptured cell membranes, and invasion of inflammatory cells into the uppermost epithelial layer were obvious in the "closed" group. In the "open" group, an increase of intercellular volume, accumulation of mitochondria in the supranuclear part of the cells; cytoplasmic protrusions, and mucosal deposits on the top of the cilia were observed (Figure 2).

Four weeks later, the amounts of nasal secretion and sinus pus had decreased significantly in the "open" group. In the "closed" group, severe oedema, swelling, hyperaemia and partial necrosis of mucosa were observed (Table 2). Light-

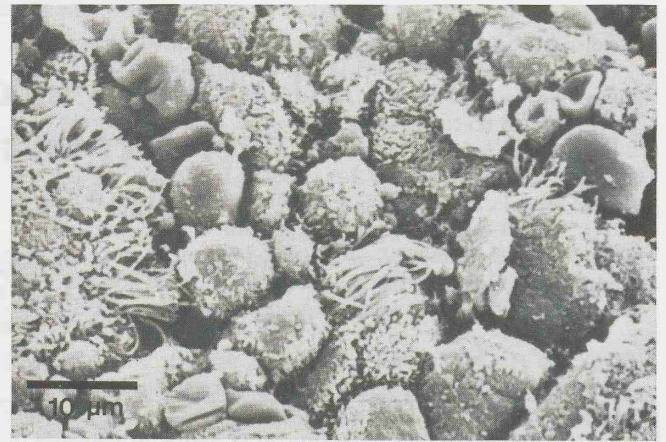


Figure 1. Scanning electron-microscopical findings in group I (2-week "closed" group). The number of ciliated cells is considerably decreased, the shape of the cilia is disturbed, and compound cilia are observed.



Figure 2. Transmission electron-microscopical findings in group III (2-week "open" group). Invasion of inflammatory cells (NT: neutrophil; LY: lymphocyte) into the middle part of the epithelial layer is observed. A tendency to expansion of the intercellular space is noted (small arrow). Accumulation of mitochondria (arrow head) found in the supranuclear part. Compound cilia of the "adhesive" type (closed arrow) and "bulging" type (open arrow) are observed.

Table 2 Amount of nasal discharge and secretion in the maxillary sinus on the 33rd experimental day (N=16 rabbits).

gross findings	group II (N=8)	Group IV (N=8)
<i>nasal discharge:</i>		
absent	0	2
mild	5	5
moderate	3	1
<i>secretion in sinus:*</i>		
absent	0	1
mild	0	3
moderate	1	2
severe	7	2

\*: The amount of secretion in the sinus was significantly more decreased in group IV than in group II ( $p < 0.05$ ).

Table 3 Lightmicroscopical findings of the maxillary sinus mucosa on the 33rd experimental day (N=16 rabbits).

pathologic findings	group II (N=8)	group IV (N=8)
<i>cilia,y loss:*</i>		
absent	0	1
mild	1	3
moderate	1	2
severe	6	2
<i>epithelial ulceration:*</i>		
absent	0	2
mild	1	2
moderate	2	3
severe	5	1
<i>infiltration ofinflammato,y cells:*</i>		
absent	0	1
mild	0	4
moderate	3	2
severe	5	1
<i>oedema and congestion:</i>		
absent	1	2
mild	3	3
moderate	4	3

\*: The degrees of ciliary loss, epithelial ulceration, and infiltration of inflammatory cells were significantly less in group IV than in group II ( $p < 0.05$ ).

microscopically, epithelial ulceration, loss of cilia, oedema and congestion were severe and, in addition, fibrosis and squamous metaplasia were found in the "closed" group. The degree of inflammation had significantly decreased more in the "open" group than in the "closed" group (Table 3), but there was no significant improvement between group III (2-week "open" group) and group IV (4-week "open" group). Ciliated cells were markedly decreased, and compound cilia and disturbed shape of the cilia were observed in the "closed" group (Figure 3). But in the "open" group ciliary loss was minimal, and the stiffness or orientation of the cilia looked normal (Figure 4). At the ultrastructural level, more severe changes were found in the "closed" group. The ciliated epithelia had nearly changed to non-ciliated epithelia, the cell membranes were locally ruptured, and the intercellular spaces were more prominent (Figure 5). Neutrophils, plasma cells,

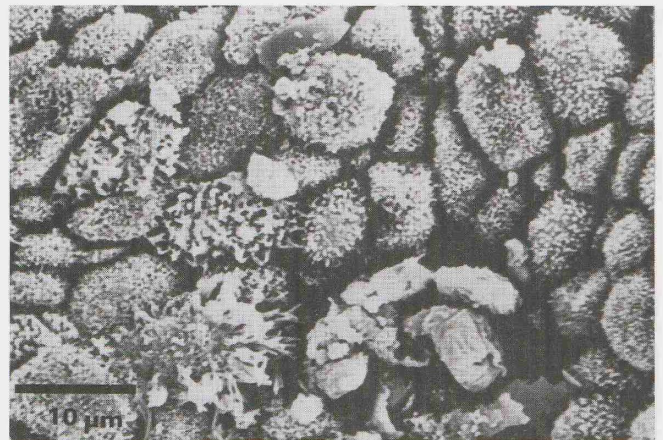


Figure 3. Scanning electron-microscopical findings in group II (4-week "closed" group). Ciliated cells are significantly decreased in number in this area. Compound cilia are found, and the shape of the cilia is disturbed.

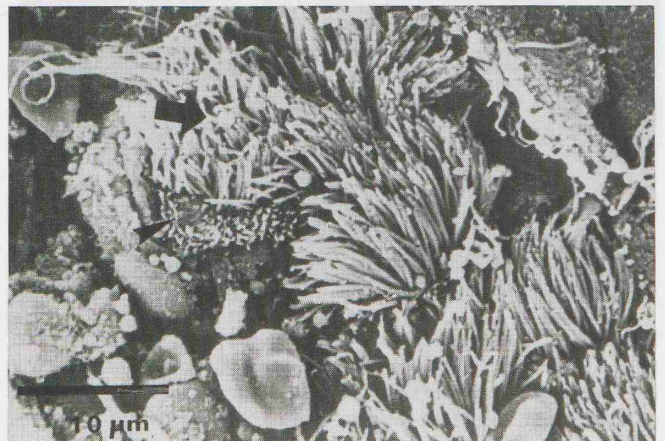


Figure 4. Scanning electron-microscopical findings in group IV (4-week "open" group). Ciliated cells are decreased, but the shape and orientation of the cilia are normal. A deposit (arrow) of mucus and a protrusion of cytoplasm (arrow head) are noted on the cell's apical surface.

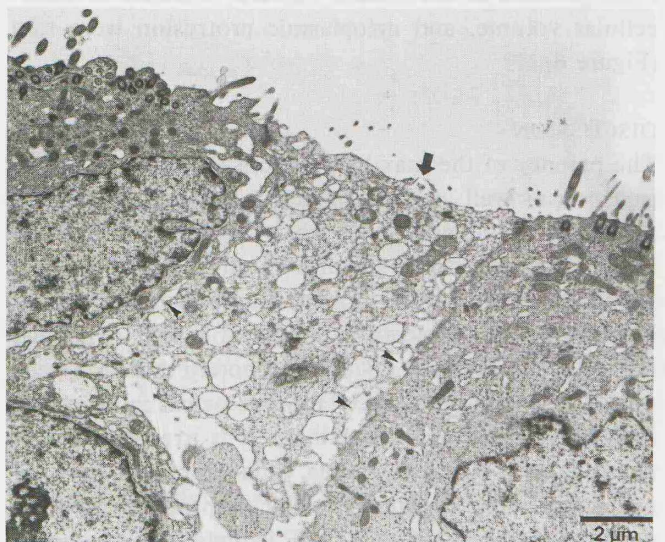


Figure 5. Transmission electron-microscopical findings in group II (4-week "closed" group). Few ciliated cells are found in this area. Focal rupture of the cell membrane (arrow) is observed. Expansion of the intercellular space is more extensive (arrow head).

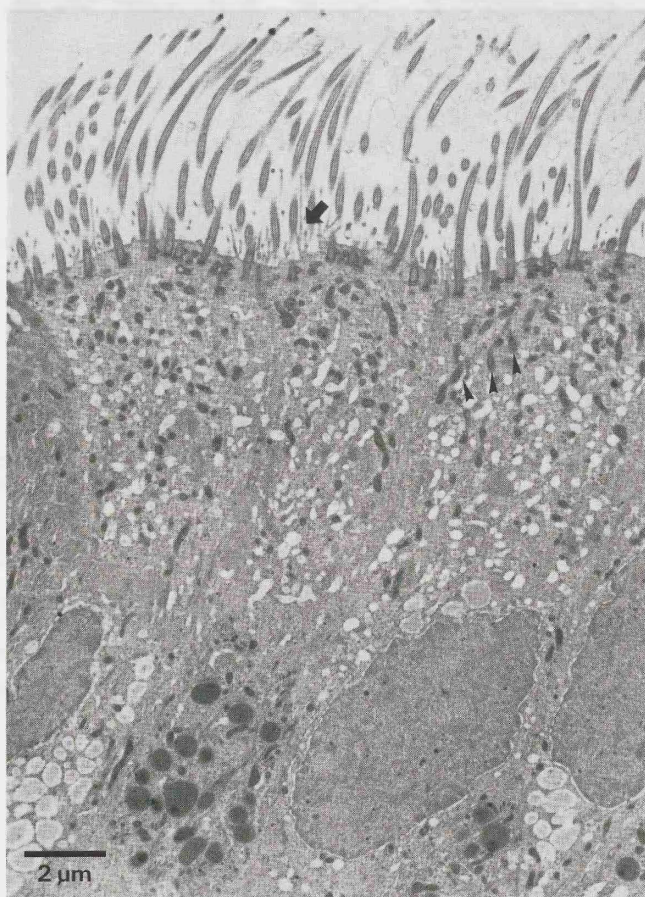


Figure 6 Transmission electron-microscopical findings in group IV (4-week "open" group). Accumulation of mitochondria (arrow head) is found in the supranuclear part. On the cell surface, an increase in long and branched microvilli (arrow) is noted.

lymphocytes and epithelial cells with tonofilaments were observed. In contrast, in the "open" group the epithelial layer was mainly composed of ciliated cells, and abnormal findings such as membrane rupture, increase in intercellular volume, and cytoplasmic protrusion were rare (Figure 6).

#### DISCUSSION

The patency of the maxillary ostium affects mucociliary transport as well as gaseous exchange. Secretion of the sinus mucosa is expelled through the ostium, via the mucociliary transport system, and the oxygen for sinus mucosa is supplied by ventilation. *In vitro*, when the sinus cavity is replaced by an anoxic environment, mucociliary activity rapidly ceases. Reimer (1978) has reported that prolonged anoxia results in pronounced morphological changes, and it has been observed that ciliostasis is irreversible after approximately 60 min of anoxia. Ciliary functioning requires energy in the form of ATP, which is produced by mitochondria in the living cells; ATP is replenished by either the oxidative (aerobic) or glycolytic (anaerobic) pathway (Satir, 1974).

Johansson et al. (1988) suggested that two of the most important factors in the induction of sinusitis are ostial

obstruction and bacterial infection. Lee (1991) confirmed that both ostial obstruction and inoculation of pathogenic bacteria are prerequisites for inducing experimental sinusitis. Ostial patency is our first concern, so we divided experimental sinusitis into an "open" and "closed" group. The amount of nasal and sinus secretion, sinus mucosa! swelling, and hyperaemia were more severe in the "closed" group and they worsened with the duration of the obstruction. In the study of Reimer (1978), solutions to blocking such as antral drainage and antrostomy were beneficial, possibly due to the improved gaseous environment and its positive effect on the mucociliary system.

Inflammation developed mainly in the mucous membranes of the maxillary sinuses. The changes of the mucosa included: a reduction of the ciliated surface and morphological distortion of the cilia such as compound cilia, bleb formation, and swelling of the top ends of the cilia (cf., Maeyama, 1981). According to Ohashi and Nakai (1983), the first change of inflammation in mucosa is an invasion of inflammatory cells into the epithelial layer, which is then followed by expansion of the intercellular space, vacuolation of cytoplasm, appearance of diseased non-ciliated cells, loss of ciliated cells, swelling of the ciliary membrane, loss of epithelial cells, formation of compound cilia, protrusion of cytoplasm, separation of the epithelial layer from the basal lamina, and squamous metaplasia.

In our study, histopathological findings were more severe in the "closed" group than the "open" group, and more severe in the 4-week "closed" group than the 2-week "closed" group. On the other hand, the histopathological findings had improved in both the 2- and 4-week "open" groups, but there were no significant differences between the two groups. With an opened ostium and with longer duration of this condition, the number of ciliary cells increased, destruction of the cellular membrane decreased, infiltration of inflammatory cells was reduced, and separation of the epithelial layer from basal lamina was minimized.

In conclusion, in rabbits with experimental maxillary sinusitis, the most severe histopathological findings have been observed in the "closed" group (vs the "open" group), and in the 4-week "closed" group (vs the 2-week "closed" group). We think that ostial obstruction plays a crucial role in the pathogenesis of sinusitis, and that ostial patency might have a significant effect on mucosal healing.

#### REFERENCES

1. Ballenger JJ (1985) Diseases of the Nose, Throat, Ear, Head and Neck, 13th Edition. Lea & Febiger, Philadelphia, pp. 17-23.
2. Hilding AC (1941) Experimental sinus surgery. Effects of operative windows on normal sinuses. *Ann Oto! Rhino! Laryngol* 50: 379-392.
3. Johanssen P, Kumlien J, Carlsoo B, et al. (1988) Experimental acute sinusitis in rabbits. *Acta Otolaryngol (Stockh)* 105: 357-366.
4. Kumlien J, Schiratzki H (1985) Blood flow in the rabbit sinus mucosa during experimentally induced chronic sinusitis. *Acta Otolaryngol (Stockh)* 99: 630-636.

5. Lee BJ, Kim SY, Min YG (1991) An experimental study on the pathogenesis and histopathological changes of acute maxillary sinusitis. Korean J Otolaryngol 34: 489-504.
6. Maeyama T (1981) A study of experimental sinusitis in rabbits. Auris Nasus Larynx (Tokyo) 8: 87-98.
7. Ohashi Y, Nakai Y (1983) Functional and morphological pathology of chronic sinusitis mucous membrane. Acta Otolaryngol(Stockh) Suppl 397: 11-48.
8. Reimer A, Toremalm NG (1978) The mucociliary activity of the upper respiratory tract. II. A method for *in vivo* studies on maxillary sinus mucosa of animals and human beings. Acta Otolaryngol (Stockh) 86: 283-288.
9. Reimer A (1978) The effect of carbon dioxide on the activity of cilia. Acta Otolaryngol (Stockh) 86: 156-160.
10. SAS Institute Inc (1988) SAS/STAT user's guide. Release 6.03. SAS, Cary, pp. 520-548.
11. Satir P (1974) How cilia move. Sci Am 231: 45-53.

Yang-Gi Min, MD  
 Department of Otolaryngology  
 Seoul National University Hospital  
 28, Yangon-Dong  
 Chongno-Gu  
 Seoul 110-744  
 Korea

SUMMARY

This study was designed to investigate the mucociliary clearance of the maxillary sinus in rabbits with acute maxillary sinusitis. The mucociliary clearance was measured by the fluorescent microsphere technique. The results showed that the mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group. The mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group. The mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group. The mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group.

*In vivo* mucociliary clearance in rabbits with acute maxillary sinusitis

INTRODUCTION

Maxillary sinusitis (MS) is a common disease of the upper respiratory tract and is characterized by inflammation of the mucous membrane of the sinus. The pathogenesis of MS is still unclear, but it is thought to be caused by infection, allergy, or obstruction of the sinus ostium. The mucociliary clearance of the maxillary sinus is an important factor in the pathogenesis of MS. The mucociliary clearance of the maxillary sinus is an important factor in the pathogenesis of MS. The mucociliary clearance of the maxillary sinus is an important factor in the pathogenesis of MS.

MATERIALS AND METHODS

The study was conducted in a laboratory setting. The subjects were rabbits. The study was conducted in a laboratory setting. The subjects were rabbits. The study was conducted in a laboratory setting. The subjects were rabbits.

The study was designed to investigate the mucociliary clearance of the maxillary sinus in rabbits with acute maxillary sinusitis. The mucociliary clearance was measured by the fluorescent microsphere technique. The results showed that the mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group. The mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group.

The study was designed to investigate the mucociliary clearance of the maxillary sinus in rabbits with acute maxillary sinusitis. The mucociliary clearance was measured by the fluorescent microsphere technique. The results showed that the mucociliary clearance of the maxillary sinus was significantly lower in the acute maxillary sinusitis group than in the control group.