

The effects of natural ostium and nasoantral window on mucosal regeneration after maxillary sinus surgery in rabbits*

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SUMMARY

The aims of the study were to investigate the effects of natural ostium and the nasoantral window on the mucosal regeneration after maxillary sinus surgery and to detect the changes in the mucociliary clearance during regeneration process.

Twenty-eight rabbits were studied. In the study group consisting of 21 rabbits, the mucosa of each right maxillary sinus was totally removed, natural ostium was occluded with bone-wax and a nasoantral window was created, while the left sinus mucosa was removed and the natural ostium was left open without creating a nasoantral window. Maxillary sinus mucosa taken during surgery was investigated by light and electron microscopy. The study group was divided into three subgroups. The first group was followed for 2, the second for 4, and the last group for 8 weeks. At the end of these periods, the mucociliary function was evaluated scintigraphically using ^{99m}Tc-Human serum albumin. Following this investigation mucosal biopsies were taken from the right and left sinuses and the rabbits were sacrificed. In the control group consisting of 7 rabbits a small hole was opened in the anterior wall of the maxillary sinus of each rabbit and the scintigraphic evaluation was performed for this group at the end of each follow up period as well.

In the histopathologic investigation, no difference was found between the natural and the nasoantral window using light microscopy, but there was a difference between them in the electron microscopic findings ($p < 0.05$, Chi-square test).

The scintigraphic, light and electron microscopic findings and gross appearances of the sinuses are discussed.

Key words: maxillary sinuses, nasoantral window, Caldwell-Luc operation, mucociliary clearance.

INTRODUCTION

Chronic maxillary sinusitis is one of the most common diseases seen in the upper respiratory tract. In the surgical treatment of the disease, the Caldwell-Luc operation has been used since 1887. During this operation, while some surgeons create a new nasoantral window (NW) without looking at the patency of the natural ostium (Macbeth, 1968; Mann et al., 1978; Ohashi et al., 1986; Takasaka et al., 1980), others give importance to the status of the sinus ostium which is functioning or not (Albegger, 1978; Coleman et al., 1996; Kennedy et al., 1987; Kennedy et al., 1989; Moreno et al., 1996). Functional endoscopic sinus surgery (FESS) taking the status of the natural ostium (NO) into consideration has acquired priority in the last 30 years. The differ-

ence between these two techniques is based on mucosal changes in sinusitis and the effects of NO and NW on healing mucosa after surgery. FESS has focused on the removal of gross pathologies and anatomic obstruction in the infundibular area close to the normal ostium without creating NW.

There have been different results about the functional and histological improvement of the sinus after the Caldwell-Luc operation in the literature. Some earlier reports suggested that no significant differences were noted in gross, light, or electron microscopic findings between NW and NO (Ataman, 1990; Benninger et al., 1993; Benninger et al., 1989). Some other reports claimed that the patency rate for NW was lower than others (Benninger et al, 1989; Forsgren et al, 1995). However,

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there are a few studies including investigations of both histopathologic examination of the newly regenerated mucosa and mucociliary clearance of the maxillary sinus by objective methods together (Benninger et al., 1989; Min et al., 1994; Forsgren et al., 1993). The aims of the present study were to evaluate the effects of NO and NW on the functional and histological development of mucosa in a rabbit model after maxillary sinus surgery.

MATERIALS AND METHODS

Twenty-eight New Zealand white rabbits weighting 2.4-3.0 kg were anesthetized with intramuscular injections of ketamine (30 mg/kg) and xylazine (35 mg/kg). In the study group consisting of 21 rabbits, a midline incision was made through the skin and the periosteum over the maxillary sinus. Then, the periosteum of the maxillary sinus was elevated by chisel bilaterally and the anterior wall of the sinus was entered. The mucosa of the sinus was incised. In the right sinuses, after the natural ostium was identified, it was occluded with bone-wax and a nasoantral window was created inferiorly to the natural ostium. In the left sinuses, mucosa was removed and the natural ostium was left open without creating a nasoantral window. Maxillary sinus mucosa taken during surgery was investigated by light and electron microscopy. The study group was divided into three subgroups: the first was followed for 2 (Group I), the second for 4 (Group II), and the last group for 8 weeks (Group III), respectively. At the end of these periods, infectious findings such as purulent or mucopurulent drainage were not observed in the sinus. Then, the mucociliary function was evaluated scintigraphically using ^{99m}Technetium -Human serum albumin (^{99m}Tc-HSA). Following this investigation mucosal biopsies were taken from the right and left sinuses and the rabbits were sacrificed. In the control group consisting of 7 rabbits a small hole was opened in the anterior wall of the maxillary sinus and the scintigraphic evaluation was repeated for this group at the end of each follow-up period as well.

After the scintigraphic evaluation of the study group, physiological concentrations of serum were injected through the defect of the anterior wall of both sinuses. The nasal drainage was evaluated and biopsies were taken from the newly regenerated mucosa for histopathological evaluation.

Scintigraphic evaluation:

Mucociliary clearance of both sinuses was calculated with a radionuclide mucociliary clearance study at the end of the follow-up periods. The study was performed on separate days (1 or 2 days) for each sinus so the remaining radioactivity in the previously evaluated sinus did not interfere with the data of the study of the sinus evaluated later. The rabbits were reanesthetized with the method mentioned above and stabilized to the scanning table. ^{99m}Tc-HSA was used as the radionuclide agent. Immediately after the injection of 200 microcuries of ^{99m}Tc-HSA in volume of 0.2 ml into the sinus through the anterior wall where the portion of bone was removed, the study was initialized. One-minute images were obtained for 30 minutes in a

64×64 matrix with a GE Starcam 4000i gamma camera using a LEGP collimator. By drawing regions of interest to each sinus the time-activity curves were generated. The percentage of the total number of counts cleared from the sinus was calculated and all counts were corrected for decay. In all groups the rates of clearance of the radionuclide from the sinuses were calculated and mean clearance percentages for the three study groups and the control group were calculated. The formula of sinus clearance percentage is:

$$SC_{30} = 100 - (DC_{30} \times 100/C_{max})$$

where

SC ₃₀	Percentage of sinus clearance at the end of 30 minutes.
DC ₃₀	Decay corrected counts of the radionuclide at 30 minutes.
C _{max}	The initial number of radionuclide counts in the sinus during the first minute of the study.

Gross evaluation of drainage of sinus:

After the scintigraphic measurement was completed, 2 or 3 ml physiological serum was injected into both sinuses and the nasal drainage was examined. The results were expressed as: normal, decreased, or absent. Then, a biopsy was taken and the rabbits were sacrificed.

Histopathological evaluation:

Light microscopy: frozen sections of 4µm thick were stained with hematoxylin-eosin. Semiquantitative grading was performed in comparison with the normal rabbit sinus mucosal morphology according to various parameters such as ciliary development, glandular development, acute and chronic inflammation, fibrosis and epithelial hypertrophy.

Electron microscopy: specimens of all study groups and controls were collected for electron microscopic evaluation. They were minced into 1 µm slices, put into cold 3.5 % gluteraldehyde, rinsed in buffer, post-fixed in 1% osmic acid, dehydrated with alcohol. Examination was done by transmission electron microscope.

Results were analyzed using the Chi-square test.

RESULTS

I. Scintigraphic findings:

The average percentages of clearance in 30 minutes is given in Table 1. According to these results it can be seen that in all groups, no statistically significant differences between the NO and NW groups regarding percentages of clearance exist (p>0.05). However, in Group I, it can be seen that both NO and NW had a more rapid drainage than the controls (p<0.05). In addition, there are statistically significant differences between Group I on one hand and Group II and III on the other hand, whereas no differences exist between Group II and III.

Table 1. The average percentages of clearance in 30 minutes in the study and the control groups.

GROUPS	PERCENTAGES OF CLEARANCE	STANDARD DEVIATION
CONTROL STUDY	21.51±	3.15
Group I with NW	30.25±	2.85
Group I with NO	32.51±	4.58
Group II with NW	18.90±	1.90
Group II with NO	24.65±	3.35
Group III with NW	19.50±	2.51
Group III with NO	24.22±	3.41

NW: Nasoantral window NO: Natural Ostium

II. Histopathological findings:

A. Light microscopy:

Light microscopic changes of the mucosa were similar in the groups with NW and those with NO (Figure 1). No granulation tissue like the foreign body reaction opposed to bone-wax has been observed.

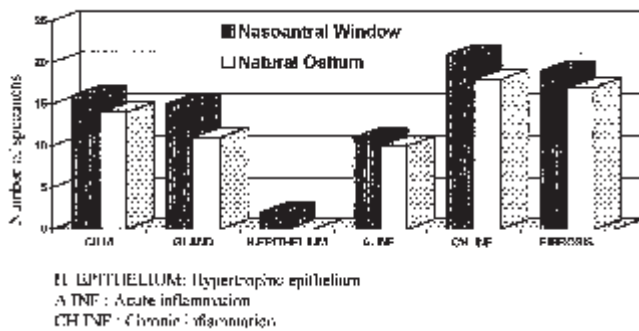


Figure 1. The distribution of differences between histopathological findings of the nasoantral window and the natural ostium groups at the end of the follow-up periods.

- a. Ciliary regeneration: in Group I, regular ciliary structure has been seen in both the NW and NO groups, but 6 (42.8%) specimens showed little and abnormally appeared ciliary epithelium. In Group II, although ciliary epithelium was normal in 7 (50%) of the specimens and abnormal in 4 (28.57%), there were no cilia in 3 (21.42%) of the specimens. In Group III, 10 (71.4%) of the specimens in the NW and NO groups demonstrated a normal ciliary structure. Furthermore, there were abnormal cilia in 3 (21.42%) of the specimens and no cilia in 1 (7.1%) of the specimens. The findings between the NW and NO groups showed no statistically significant difference with respect to the regeneration of cilia in all periods postoperatively ($p>0.05$).
- b. Seromucinous glandular regeneration: in Group I, 9 specimens of both sinuses including the NW and NO groups had

no glandular structure, but in 5 specimens a few glands were seen. In Group II, only 3 specimens in the NO group had regular epithelium with intact seromucinous glands. However, in 7 specimens of both the NW and NO groups little and untidy glands could be observed. In Group III, in 3 specimens of both NW and NO, the glandular epithelium had been regenerated regularly. Regarding the gland regeneration there was no statistically significant difference between NO and NW at 8 weeks postoperatively ($p>0.05$). However, there were statistically significant differences between Group II and III ($p<0.05$).

- c. Hypertrophic epithelium: only 2 specimens had hypertrophic epithelium 8 weeks postoperatively. Therefore, no statistical analysis could be performed.
- d. Acute inflammation: although the NO and NW groups showed no difference statistically in all groups, acute inflammation was much more common in the mucosa of NW (50%) compared to the NO groups (28.2%) in Group II and III.
- e. Chronic inflammation: in all groups, it was seen that chronic inflammation was commonly focal in nature. It was much more common in the mucosa of NW (64.2%) than in the NO groups (35.8%), especially in Group II and III.
- f. Fibrosis: Fibrosis was similar between NW (90.4%) and NO (80.9%) mucosa specimens of all groups. This fibrosis was limited to the submucosal area and the volume of the sinus cavity did not change.

B. Electron microscopy:

In Group I, 2 representative samples of regenerated sinus mucosa from both the NO and NW groups showed compound and oedematous cilia but no alterations in the cilia microtubules (normal 9+2 microtubular arrangement) was observed. In Group II and III, cilia taken from the NO group were normal, but samples from the NW group showed compound cilia (Figures 2 and 3).

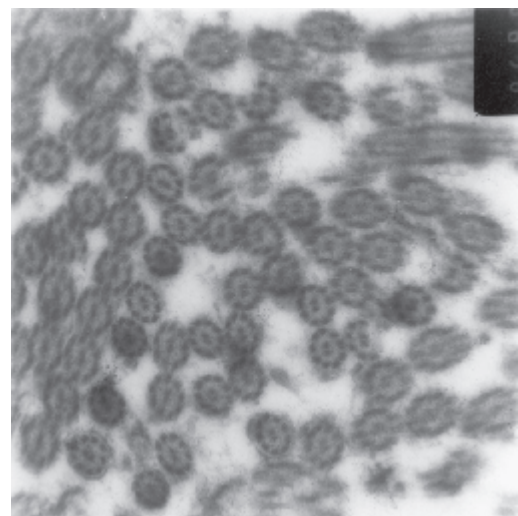


Figure 2. Abnormal cilia including compound cilia in the nasoantral window groups, 8 weeks postoperatively (original magnification $\times 27000$).

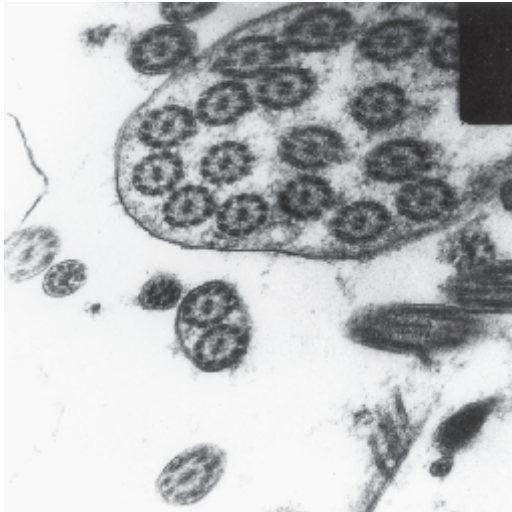


Figure 3. Normal cilia and normal 9+2 microtubule arrangement in the natural ostium groups, 8 weeks postoperatively (original magnification $\times 27000$).

III. Gross evaluation of the sinuses and drainage examination with serum physiologic:

After 4 weeks, most of the sinuses were obliterated with fibrous tissue and new bone formation. There was granulation tissue especially around the NW and in some cases at 8th weeks, the NW was obstructed. However, the fibrosis and new bone formation were limited to the NW and around it. There was no gross obliteration in the sinus cavity.

Beginning from the 4th week, drainage from the NW in most of the sinuses decreased gradually when compared to the NO.

DISCUSSION

Surgical management of chronic maxillary sinusitis was performed by removing the mucosa, either partially or totally, and creating a new nasoantral window performed by the Caldwell-Luc procedure. Some authors have suggested that all of the sinus mucosa needs to be removed as there could be submucosal microcysts in the mucosa which appears normal (Macbeth, 1968). Takasaka et al. (1980) recommended that pathologic mucosa has to be removed because of the development of atypical cilia and a decrease of cilia motility in the chronic maxillary sinusitis.

Forsgren et al. (1995) showed that in the Caldwell-Luc approach in which the mucosa is entirely or partially removed, and an inferior meatal window is created, there was a significant decrease in subepithelial thickening, inflammation, oedema, polypoid formation and submucosal gland but an increase in fibrosis apparently in the postoperative period when compared with the preoperative period.

Moreno et al. (1996) investigated the changes in the submucosal glands after complete or partial surgical removal of the maxillary sinus mucosa in rabbits. They reported that a decrease in the number of serous glands and significant inflammation was present in the sinus in which the mucosa was completely removed.

In a study by Forsgren et al. (1995), in which the Caldwell-Luc surgery was performed on the animals, was reported that the mucosa was reepithelized within two weeks. In the same study, regeneration of the lamina propria was incomplete and reactive cellular processes such as bone remodeling, fibroblast proliferation, formation of polyps and atypical glands were characteristics of regenerated the mucosa. After 2 weeks, a further differentiation towards the ciliated epithelium took place and the epithelium had a normal structure at 6 and 9 months postoperatively. Benninger et al. (1993) compared the ciliary epithelium in the NW with NO groups after sinus surgery and found that the ciliary epithelium in the NW group was normal compared to the NO group.

In the present study, although only a few ciliary structures were present in both groups at the second week, there was completely normal ciliary epithelium in the ratio of 43% and 57% in the NO and NW groups, respectively, at the end of the first month. Furthermore, normal ciliary epithelium was found in the ratio of 71% in both groups at the end of 2 months. Most of the sinuses were lined with ciliary epithelium in both groups after 8 weeks. At the end of 8 weeks, more seromucinous glands were observed in the NO group than in the NW group with respect to the glandular structure investigation.

Hosemann et al. (1991a; 1991b) investigated wound closure in rabbits and reported that wound closure started 120 hours after the granulation tissue covering the wound surface started to become hyperplastic, and bone apposition with formation of osteoid occurred. Animals in our study were sacrificed after 2 weeks at the earliest. Granulation tissue as an acute phase of wound healing would be terminated in this period. Instead, we observed fibrosis which is the result of morphological changes after 2 weeks. This fibrosis was limited to the submucosal area. In an electron microscopic investigation, Albegger (1978) reported that a mucosa, including abnormal cilia that were longer and thinner, had developed after the Caldwell-Luc operation. Min et al. (1994) investigated the cilia structure in the regenerated mucosa at the 8th week after the Caldwell-Luc operation and found about 10% oedematous cilia, compound cilia and a degeneration of the 9+2 microtubular arrangement. Using the electronmicroscope in the present study, it was revealed that compound cilia without deviations from the 9+2 microtubular arrangement in the NW and NO groups at 2 weeks postoperatively. Although the ciliary epithelium in the NO group was normal, and the ostium was patent, abnormal cilia were still present at the end of the 8th week in the NW group (Figures 1 and 2).

In the literature there are different reports about the changes of the sinus function and the effect of NW on this function after surgery. Mann et al. (1978) reported in their patients that had undergone the Caldwell-Luc operation that NW provided the mucociliary drainage during 2 years postoperatively. In the same study, it was also reported that NW did not affect the rate of recurrence and reinfections. In another study, the drainage of the maxillary sinus has been investigated by a scintigraphic method after the Caldwell-Luc operation and it was reported that although the drainage of the NO when the mucosa totally

removed, was lower than in NW, but there was no difference between NO and NW in cases with an untouched mucosa (Ataman, 1990). As a result of this, it has been assessed that a NW should be created in cases with a totally removed mucosa, as regenerated mucosa would not be able to provide mucociliary activity.

In a study done to compare the patency of NO and NW of rabbits where the mucosa was totally removed using Caldwell-Luc surgery, physiological amounts of serum were given into the maxillary sinus 8 weeks postoperatively (Benninger et al., 1993). The patency was 71% and 78% in the NO and NW groups, respectively. In the present study, the flow rate was nearly 100% in both the NO and NW groups 2 weeks after the operation, but decreased slightly in the NW starting by the 1st month, and this became apparent during the 2nd month.

Friedman and Toriumi (1989) investigated the effect of NW on mucociliary clearance and reported that the mucociliary clearance was impaired when the sinus mucosa was removed. However, there was no difference between the study and control groups regarding a created NW and untouched mucosa. The present study showed that although there was a rapid drainage in the early period of regeneration, the clearance could return to normal only in the cases when the regeneration of the cilia and the seromucinous glands was completed. The reasons for the rapid drainage in the early period could be that there was no glandular regeneration, the mucus was very viscous and the epithelium in the sinus was very thin. It has been observed that mucociliary activity was nearly normal as the ciliary and glandular structure reached towards normal levels in the NO group. In contrast, in the NW group, although the ciliary and glandular structures developed gradually, the mucociliary clearance decreased under normal levels starting at the beginning of the 4th week. The present study indicated that mucosal regeneration began following surgery and irregular ciliary structure was observed in the 2nd week but reached to normality not earlier than at the end of the 4th week. In addition, seromucinous glands could be observed in low numbers at the end of the 4th week, but reached normal numbers at the end of the 8th week. According to our results, both NW and NO have similar effects on the mucosal regeneration after removal of the mucosa when inflamed mucosa was excluded. When the development of cilia and seromucinous glands did not return to normal, the sinusitis will reappear. In our study this period is found to be approximately 8 weeks in rabbits. During this period, only low mucus production occurs inside the sinus due to an incomplete regeneration of the mucosa and this will create an appropriate environment for bacterial growth. A reaction will take place before the development of a normal ciliary function. There is concomitance between mucosal regeneration and a return of the mucociliary function. After a clear period from the beginning of the regeneration, cilia will start functioning and the drainage of the sinus will begin actively. If there is a problem in the regeneration of the mucosa or ventilation of the sinus, drainage of the sinus will decrease gradually. It is possible to detect the mucociliary activity of the sinus and to measure the effects of the surgical procedure by the ^{99m}Tc -HSA scintigraphy. According to

our results, a NW created during the Caldwell-Luc surgery closes after a short period and the mucociliary drainage is impaired because of a decrease of sinus ventilation. If the cilia and seromucinous glands do not develop, mucus will not drain and an appropriate environment for bacterial growth will develop. The reason for the NW to close in a short time is the narrowing of the ostium because of mucosal inflammation.

CONCLUSION

The present study indicates that there was no difference in effects on mucosal regeneration of NW and patent NO after maxillary sinus surgery. Mucosal maturation was also completed in both procedures. However, this process will take a long time. The maxillary sinus has a tendency for reinfections because cilia will not work properly. Therefore, one should be careful with the patency of the natural ostium and one should observe whether the mucosa is healthy before deciding what kind of operation has to be performed. In the cases with an unhealthy sinus mucosa and if the natural ostium is not patent, one should be assured that it is functioning, because the naso-antral window alone cannot manage the sinus function sufficiently.

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