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Experimental sinusitis in nasally catheterised rabbits*

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SUMMARY

Aim: The aim of the study was to create an experimental rabbit model for investigating the effects of nasal catheterization on rhinosinus mucosa, bacterial flora and observing the development of bacterial sinusitis.

Methods: Healthy adult white rabbits of either sex and with body weights of 2,5-3 kg were used. Rabbits were randomly separated into two groups; the first group was catheterized by 12 French and the second group was catheterized by 8 French catheters blindly and the non-catheterized left sides were accepted as control. Three randomly chosen rabbits from each group were examined by computerized tomography scans (CT) and sacrified in the first, second and the fourth week of the study. Microbiological and histopathological examinations were performed.

Results: In both study groups after the first week of nasal catheterization, opacity or air-fluid level was detected in maxillary sinuses by CT scans, which was significant in group 1. Inflammation spread by the prolongation of nasal catheterization and rapidly development of sinusitis was observed by thicker catheters' usage.

Conclusion: In this study, the role of nasal catheterization as a predisposing factor in the development of sinusitis and the increase of sinusitis development risk in relation with the catheterization period and the catheters' thickness was shown.

Key words: nasal catheterization, sinusitis, experimental, rabbit

INTRODUCTION

Nosocomial sinusitis is an important origin of nosocomial sepsis or pneumonia in intensive care units where the critically ill patients are hospitalized. The risk of maxillary sinusitis has increased especially in nasally catheterized patients. Nasal catheterization is a predisposing factor of inflammatory reaction in nasal mucosa by causing a continuous trauma as in the case of foreign bodies. Spreading of edema in the maxillary ostium and meatus leads to growth of bacteria. This results in bacterial maxillary sinusitis development in the region adjacent to the nasally placed catheters (Linden et al., 1988; Borman et al., 1992; Bert et al., 1996; Talmor et al., 1997; George et al., 1998; Ramadan et al., 1998; Holzapfel et al., 1999).

Experimental animal models are useful to study the different aspects of the pathogenesis of various diseases. For sinusitis, rabbits are the mostly studied experimental animals (Johansson et al., 1988; Westrin et al., 1990; Hinni et al., 1992; Fukami et al., 1993; Marks, 1998). In order to investigate the effects of nasal catheterization on the rhinosinus mucosa and bacterial flora and to observe the development of bacterial maxillary sinusitis, a rabbit model was developed.

MATERIALS AND METHODS

Eighteen healthy adult New Zealand white rabbits of either sex and with body weights of 2,5-3 kg were used. Free access to water and standard pelleted food was available throughout the experiment. All procedures that were carried out on animals were conducted in compliance with national and local regula-

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Table 1. Results of presacrification computerize tomography scans of rabbits.

	Group 1 (RS)		Group 2 (RS)	
Week	Unilateral	Bilateral	Unilateral	Bilateral
1st week	1	1	1	-
2nd week	2	1	1	-
4th week	1	2	2	1

RS: Radiographic Sinusitis (air-fluid level or complete opacification of maxillary sinus by CT examinations)
Unilateral: Radiographic sinusitis in right maxillary sinus,
Bilateral: Radiographic sinusitis in both maxillary sinuses.

tions and institutional guidelines for humane use of animal research. The animals were anaesthetized by an intramuscular injection of 50mg/kg ketamin hydrochloride and 10mg/kg xylasine hydrochloride before all surgical and radiological procedures.

Maxillary sinuses of rabbits were examined by a computerized tomography (CT) scan (X-Vision Spiral CT scanner, Toshiba, Japan) before the nasal catheterization and before sacrification. Coronal sections were obtained 1 cm behind the beginning of the animal's tip to the posterior wall of the maxillary sinus. Sections were obtained perpendicular to the hard palate with a 3 mm thickness, 1 increment (continue), matrix size: 512x512, 230 mAs and no interval at the bony window (ww: 2300, wl: 450). The rabbits were evaluated with coronal CT for the development of maxillary sinusitis. The existence of an air-fluid level or complete opacification of the maxillary sinus by CT examinations was regarded as radiographic sinusitis (RS) (Borman et al., 1992). The subjects were divided into two groups: 12 French (outer diameter is approximately 3.96 mm) catheters for the first group and 8 French (outer diameter is approximately 2.74 mm) catheters for the second group were applied blindly into the right nasal cavity. The catheters were 5 cm. piece of suction catheters of polyvinyl chloride (B°çakc lar, Istanbul, Turkey). After the catheterization, catheters were sutured to the mucosa of the anterior part of the nose with 4.0 silk suture material.

Three rabbits were chosen randomly from each group at the end of the first, second and fourth week. After CT examinations, animals were sacrificed by a lethal dose of sodium pentobarbital that was administered via the intracardiac route. After sacrification, the maxillary sinuses and nasal cavities of the animals were explored macroscopically to observe the contents of the sinuses and the nasal cavity, and the findings were recorded. Microbiological samples were obtained from each sinus and nasal cavity by sterile cotton swabs and placed in Stuart transport medium. All culture samples were immediately transported to the microbiological laboratory. In the laboratory, after the microscopic examinations, aerobic, anaerobic and mycological cultures were performed. The isolation and identification of the microorganisms were carried out according to the

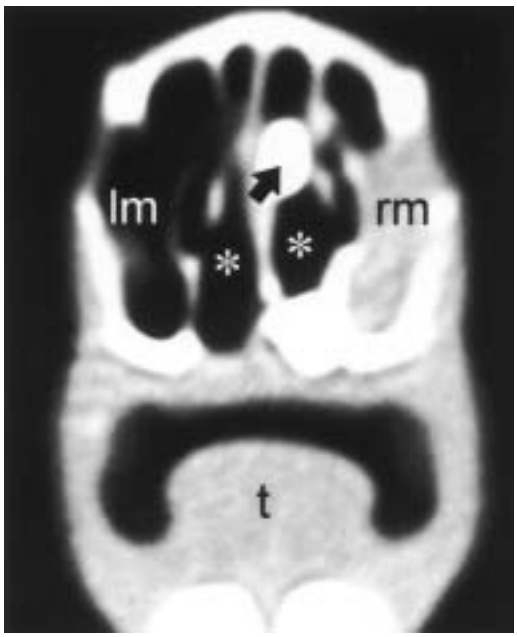

Figure 1. Air-fluid level in right maxillary sinus which was detected by coronal CT (first week, group 2). Arrow shows the catheter. A radio-opaque fluid (lipiodol®) was injected into the catheter to provide a better view of catheter localization. Radio-opacity on the floor of the nasal cavity appeared as a result of lipiodol leakage into the nasal cavity. (lm: left maxillary sinus; rm: right maxillary sinus; t: tongue; *: nasal cavity).

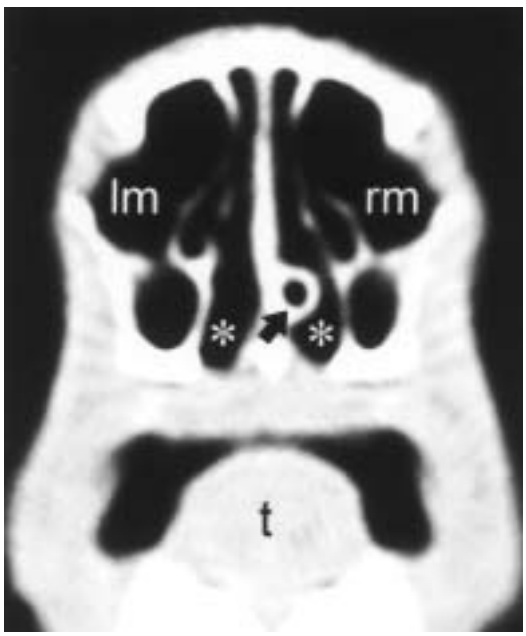

Figure 2. Catheter placed on the inferior part of the nasal cavity and leaning against the septum is seen in the right nasal cavity. Arrow shows the catheter. There was no opacity or air-fluid level in the sinuses (first week, group 2). (lm: left maxillary sinus; rm: right maxillary sinus; t: tongue; *: nasal cavity).

standard microbiological procedures.
After decapitation and dissection, the specimens were fixated in buffered 10% formaldehyde and than decalcified by 10% formic acid. Paraffin-embedded tissue sections of 5 mm thickness containing the right and left maxillary sinuses and nasal cavities were prepared. These sections were stained with haematoxylin and eosin with periodic acid schiff and alcian blue dyes.

RESULTS
When the rabbits were evaluated by coronal CT scans before the nasal catheterization, neither the existence of air-fluid level nor the opacity in maxillary sinuses was detected. Presacrification CT scan results of both groups of rabbits are shown in Table 1. In group 1, while unilateral RS was detected in the right maxillary sinuses in two out of three rabbits in the first week, whereas in the following weeks, unilateral or bilateral RS developed in all rabbits. In group 2, in both the first and the second week of the study, one of three rabbits had unilateral RS. In Figures 1 and 2, first week CT scans of two rabbits from group 2 are shown. In the fourth week, two out of three animals had (one unilateral, one bilateral) RS in group 2. In both groups, spreading of the inflammation to the left side was more significant by the prolongation of the catheterization.

In macroscopic examinations, all rabbits had secretions in and/or adjacent to the catheters. Edema and local redness were seen on the mucosa of nasal cavities. Macroscopically observed profuse purulent secretions from the maxillary sinuses always correlated to radiologically determined sinusitis. The rabbits with bilateral RS usually had septal perforations in their macroscopic examinations.

In microbiological examinations, while *Escherichia coli* had been isolated as a predominant microorganism in cultures of group 1 in the first week, in the following weeks of study, other Gram negative bacilli like *Pseudomonas aeruginosa, Proteus vulgaris, Alcaligenes* spp. were also found. In group 2 in the first week the isolated bacteria were similar to group 1, but later the microbiological spectrum changed with the predominance of Gram positive bacteria. In the study period, no anaerobic bacterial growth was detected in any culture. Results of microbiological examinations are shown in Table 2.

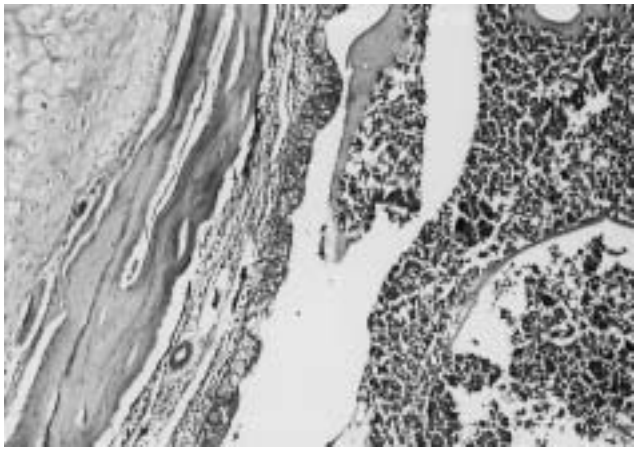

Figure 3. Purulent exudate which contains the acute inflammatory cells in the nasal cavity. Polypoid appearance of superficial epithelium and inflammatory cells in the lamina propria in the first week (100x, H&E).

In histopathological examinations, at the end of the first week, when the nasal mucosa of rabbits with RS were investigated, severe inflammation with polymorphonuclear leukocytes infiltration and edema were detected. A polymorphonuclear leukocyte infiltration in the nasal cavity of one of the rabbits is shown in Figure 3. When the nasal mucosa of rabbits without RS was investigated, focal inflammation of mucosa, minimal edema and areas of cilia loss in the right nasal cavities were detected.
After the first week, similar histopathologic changes were observed in the sinus and nasal cavities of rabbits that had RS in both groups. Polymorphonuclear leucocyte infiltration, epithelial desquamation, and squamous cell metaplasia were the most obvious findings in the sinus and nasal mucosa in the

Table 2. The microorganisms which isolated from the nasal cavity and sinuses of the rabbits.

	Group 1			Group 2		
Bacteria	**1st week R/L**	**2nd week R/L**	**4th week R/L**	**1st week R/L**	**2nd week R/L**	**4th week R/L**
E.coli	3/3	3/3	2/3	3/3		
P.vulgaris		1/0	1/1			
P.aeruginosa		2/0	1/1		2/2	1/1
Enterococcus spp.					1/1	
α-hemolytic streptococci					1/1	2/2
Coagulase negative staphylococcus				1/1	0/1	3/3
Klebsiella spp.				0/1		
Alcaligenes spp			1/0			

R: Right nasal cavity and sinus L: Left nasal cavity and sinus

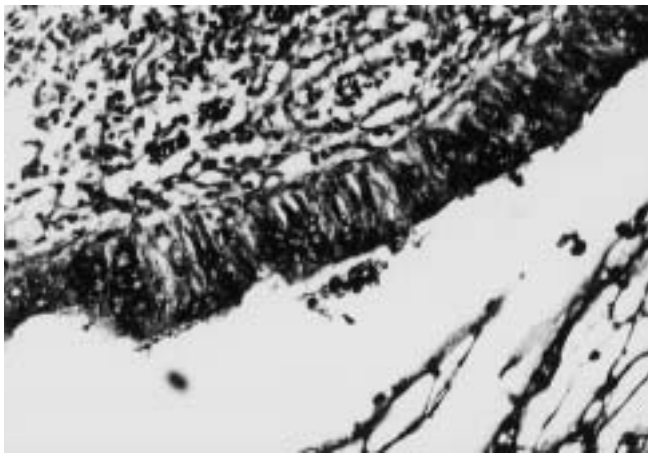

Figure 4. Epithelial hyperplasia and goblet cell increase of the sinus mucosa in the fourth week (400x, PAS-AB).

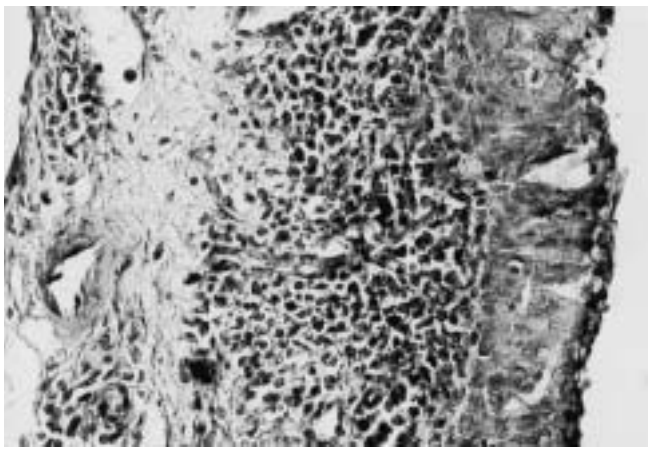

Figure 5. Epithelial hyperplasia, loss of cilia and chronic inflammatory reaction in the fourth week (400x, H&E).

second week. At the fourth week of study, chronic inflammatory reactions, characterized by eosinophil leucocytes and mononuclear cell infiltration, were seen. A significant increase of goblet cells, epithelial hyperplasia, loss of cilia and chronic inflammatory reaction as seen in the fourth week is shown in Figures 4 and 5. In the fourth week, hypertrophied lymphoid follicules, fibrosis, bone changes like a periosteal reaction and osteoneogenesis had also developed. Since the first week, polyp formations were detected in all of the right nasal cavities and in some of the sinus mucosae (Figures 3 and 6).

DISCUSSION

In human, the diagnosis of nosocomial sinusitis is usually based on radiological examinations, by radiograph or CT scan, and detection of maxillary sinus opacification or air-fluid level has to be supported by microbiological cultures of the maxillary sinus aspirate (Linden et al., 1988; Bormann et al., 1992). CT scans give an accurate and detailed picture of the paranasal sinus anatomy and pathology. Özüer (1998) examined the radiological anatomy of rabbit sinuses by CT scans and determined that CT was a reliable tool to be used in experimental models of sinusitis. Kerschner (2000) compared CT with magnetic resonance imaging in an acute sinusitis rabbit model and it was concluded that greater interobserver consistency of scan interpretation made CT as the preferred tool for diagnosing sinusitis with less time and cost.

Borman et al. (1992) accepted air-fluid level and opacifications as major CT findings to diagnose sinusitis that occurs due to nasal intubation. Therefore, we used the same criteria as Borman et al. (1992) in CT investigations and compared the results of the radiological and macroscopic examinations of the animals. While more or less secretions were detected in all nasal cavities adjacent to the catheters regardless of the radiological findings of the maxillary sinuses, profuse purulent secretions always correlated with RS.

Additionally, radiological examinations may also be useful to explain the mechanism of sinusitis development due to nasal catheterization. Localization of the tube in the nasal cavity may be an important predisposing factor for the induction of sinusitis. Compression of the middle turbinate against the lateral wall, the sinus openings with congestion and edema, will block the openings (Collins et al., 1993). In our study, Figures 1 and 2 show evidence to support this mechanism. Both figures are CT scans of group 2 rabbits that were sacrified at the end of the first week. In Figure 1, a catheter was placed between the middle turbinate and the septum which resulted in the occurrence of air-fluid level. On the other hand, when the catheter was placed in the lower part of the nasal cavity as seen in Figure 2, no radiologic evidence of sinusitis was detected.

Westergren (1999) described three phases of the inflammatory reaction that were caused by nasal intubation. In phase 1, a tube caused local mucosa injury and colonization of microorganisms. This was an initial reaction to an foreign body and resulted in polyp formation and fibrosis in the mucosa. In phase 2, functional impairment was developed and increments of secretions, changes in mucocilliary clearance and a local immune response were seen. In phase 3, an increase of local mucosa pathology was detected. It is difficult to categorize our

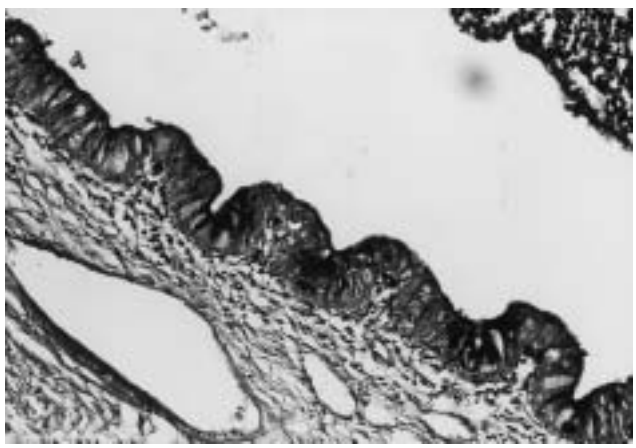

Figure 5. Epithelial hyperplasia, loss of cilia and chronic inflammatory reaction in the fourth week (400x, H&E).

results into three phases, but all described characteristics of each phase were determined in this study.

In this study, the importance of the catheters' thickness in the development of sinusitis was also emphasized. Incidence of RS in the rabbits of group 1 was more than the incidence of RS in group 2 during the study. Additionally in the fourth week, spreading of inflammation to the opposite sinuses was shown especially in group 1 which were catheterized by thicker catheters. Usage of thicker catheters not only increased the risk of sinusitis development but also caused the inflammation to spread to opposite sides after due to a developed septal perforation. Detection of bilateral maxillary opacities was always correlated with septal perforation and became more significant by the prolongation of nasal catheterization. Septal perforation and nasal necrosis are the complications of nasal intubation also in human and occur mostly with chronic intubation (Collins, ed, 1993; Stone and Gal, 2000).

Nonspecific acute or chronic inflammatory changes, goblet cell increase, epithelial desquamation, squamous cell metaplasia, gland involution, bone remodeling and polyp formations are the characteristic histologic findings of experimentally induced sinusitis in rabbits (Johansson et al., 1988; Westrin et al., 1990; Fukami et al., 1993; Hinni et al., 1992; Marks, 1998). Similar histopathologic changes that show the evidence of sinusitis were also determined in the current study.

Development of mucosal injury due to nasal intubation resulted with microorganism growth in the nasal cavity and maxillary sinuses. The isolated bacteria from the unilateral nasal cavity and sinus cultures were usually found to be similar to each other. As bacterial growth was detected with or without the evidence of RS, isolation without RS was accepted as colonization according to Westergren et al. (1999).

They studied the effects of intubation on the nasal cavity and the sinus. In their study; although inflammatory mucosa changes and increase of bacterial growth were shown in the intubated nasal cavities, the corresponding sinuses had only an increase of bacterial growth which was accepted as colonization. Determination of inflammatory changes only in the intubated side of the nasal cavities but not in sinuses, could be explained by their use of thinner catheters.

In our study, the role of the nasal catheterization as a predisposing factor in the development of maxillary sinusitis and the increase of sinusitis development risk in relation with the catheterization period and the catheters' thickness was shown. When both groups of rabbits were compared to each other it was shown that in the applied group that was treated with a thicker catheters, inflammation developed which also spreaded more rapidly and a higher number of rabbits was involved.

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