Developing A New Model for Rhinosinusitis: Animal Experiment*

Başak ÇAYPINAR**, Mustafa Salih CANPOLAT***, Pembe Gül GÜNEŞ****, Arzu İRVEM*****,
Sema ZER TOROS******, Aslı ŞAHİN YILMAZ*******, Çağatay OYSU********

Abstract

Aim: To develop a new model of acute bacterial rhinosinusitis.

Method: We divided guinea pigs into four groups in this study. In the primary group of guinea pigs, right nasal cavities of the animals were vaccinated with Streptococcus pneumoniae suspension. In the second group, sponge strips saturated with sterile saline into the right nasal cavities of animals. In the third group, sponge strips saturated with Streptococcus pneumoniae suspension in the right nasal cavities of animals. The fourth group was control group, which was exposed to no intervention. After 7, 14, 28, 35 days from interventions, intranasal cultures were obtained, computed tomography (CT) scans were imaged from all of the guinea pigs, and two randomly selected guinea pigs that were detected as having rhinosinusitis radiologically were killed every week. The sinuses and nasal specimens of sacrificed guinea pigs were prepared for histopathological investigation.

Results: Radiological and histopathological examinations of nasal samples were performed to observe the severity of the inflammatory reaction. Acute bacterial rhinosinusitis was induced in all groups of subject animals except the control group. More severe inflammation was seen in the third group of subject animals

Özgün Araştırma Makalesi (Original Research Article)
Geliş / Received: 25.05.2023 & **Kabul / Accepted:** 13.07.2023
DOI: https://doi.org/10.38079/igusabder.1288982

* This study which is to be produced from the graduation thesis titled as "Developing a New Model for Rhinosinusitis: Animal Experiment" belongs to Dr. Basak CAYPINAR whom completed E.N.T. assistancy at Ministry of Health, Umraniye Education and Treatment Hospital; and Prof. Dr. Asli SAHIN YILMAZ is the thesis advisor of this study. This study was presented at 35. Congress of Turkish Ear, Nose, Throat and Head & Neck Surgery and awarded as third place in verbal pronounce.

** Assist. Prof. Dr., Istanbul Gelisim University, Faculty of Health Sciences, Istanbul, Türkiye.
E-mail: bskcypnr@hotmail.com ORCID https://orcid.org/0000-0002-3782-4738
*** MD., HospitalTürk Hospital, Istanbul, Türkiye. E-mail: mustafasalihcanpolat@gmail.com
ORCID https://orcid.org/0000-0001-6163-4769
**** Assoc. Prof. Dr., Memorial Hospital, Istanbul, Türkiye. E-mail: pembe.gunes@memorial.com.tr
ORCID https://orcid.org/0000-0002-4982-5156
***** Assoc. Prof. Dr., Istanbul Provincial Directorate of Health, Kanuni Sultan Suleiman Training and Research Hospital, Istanbul, Türkiye. E-mail: arzuirvem@gmail.com ORCID https://orcid.org/0000-0003-1307-4538
****** Prof. Dr., Istanbul Provincial Health Directorate, Haydarpasa Numune Training and Research Hospital, Istanbul, Türkiye. E-mail: semazertoros@yahoo.com ORCID https://orcid.org/0000-0003-3125-732X
******* Prof. Dr., Istanbul Provincial Directorate of Health, Kartal Dr. Lutfi Kirdar City Hospital, Istanbul, Türkiye.
E-mail: aslisahin@hotmail.com ORCID https://orcid.org/0000-0002-7846-9453
******** Prof. Dr., Marmara University, Istanbul, Türkiye. E-mail: coysu@hotmail.com ORCID https://orcid.org/0000-0002-6756-8456

ETHICAL STATEMENT: All animal experiments were performed in compliance with a protocol number (2012/78 -ethic committee approval decision no and date) approved by the Institutional Animal Care and Use Committee of Istanbul University in 2012.

compared to the first and second groups. No inflammatory reaction was found in the control group. We also evaluated the normal flora of guinea pigs.

Conclusion: The guinea pig is certainly a true model for developing rhinosinusitis. Guinea pigs should be considered as an alternative model for further potential studies of genetics and inflammation, even though surgical manipulation is limited.

Keywords: Sinusitis, rhinosinusitis, guinea pig, animal experiment.

Yeni Bir Rinosinüzit Modeli Geliştirmek: Bir Hayvan Deneyi

Öz

Amaç: Yeni bir akut bakteriyel rinosinüzit modeli geliştirmektir.

Yöntem: Çalışmada kobay hayvanlar dört gruba ayrılmıştır. Birinci gruptaki hayvanların sağ nazal kavitelerine Streptococcus pneumoniae süspansiyonu damlatılmıştır. İkinci grupta, steril serum emdirilmiş merosel şeritler hayvanların sağ nazal pasajlarına pasajı kapatacak şekilde yerleştirilmiştir. Üçüncü grupta, Streptococcus pneumoniae emdirilmiş merosel şeritler hayvanların sağ nazal kavitelerine yerleştirilmiştir. Dördüncü grup ise herhangi bir girişim veya işlem yapılmayan hayvanların bulunduğu kontrol grubudur. Girişimleri takiben 7., 14., 28., 35. günlerde intranazal kültürler incelenmiş, bilgisayarlı tomografi (CT) görüntüleri tüm kobay hayvanlarından alınmış, ve radyoloji olarak rinosinüsit tespit edilen hayvanlardan iki tanesi rastlantısal olarak seçilerek her hafta dekapite edilmiştir. Sakrifiye edilen kobayların sinüsleri ve nazal spesimenleri histopatolojik olarak incelenmiştir.

Bulgular: Histopatolojik ve radyolojik incelemelerin yapıldığı nazal örneklemeler inflamatuar reaksiyonun ciddiyetini gözlemlemeyi sağlamıştır. Akut bakteriyel rinosinüzit, kontrol grubu hariç tüm gruplardaki kobaylarda indüklenmiştir. Üçüncü gruptaki kobaylarda ilk iki gruba nazaran daha ciddi bir inflamasyon gözlenmiştir. Kontrol grubunda herhangi bir inflamatuar reaksiyon bulunmamıştır. Ayrıca kobay hayvanlarının normal florası da incelenmiştir.

Sonuç: Kobay hayvanı deneysel olarak rinosinüzit oluşturmak için çok uygun bir hayvan deneyi modelidir. Kobay hayvanı cerrahi manipülasyondaki kısıtlılığa rağmen, ilerdeki potansiyel genetik ve inflamasyon çalışmaları için alternatif bir model olacaktır.

Anahtar Sözcükler: Sinüzit, rinosinüzit, kobay hayvanı, gine pig, hayvan deneyi.

Introduction

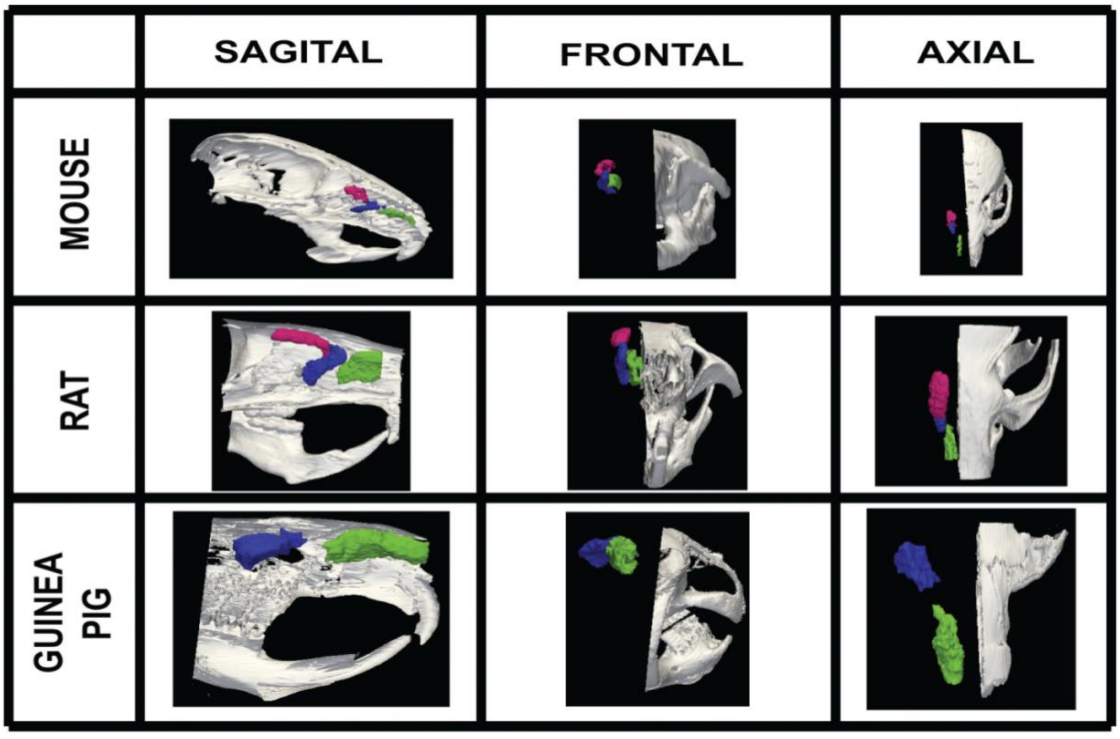
Inflammation of the mucosal edge of any of the paranasal sinuses may be the most basic definition of rhinosinusitis. Acute bacterial rhinosinusitis is among the most common diseases that need to be investigated, including the mechanism of development[1]. Rhinosinusitis has manifestations ranging from an incidental computed tomographic scan finding to an acute illness following a viral infection of the upper respiratory tract or an unremitting illness with cystic fibrosis. There is not a consensus in the literature about the pathophysiology and treatment modalities of rhinosinusitis[1]. An animal model is the best option for developing and mimicking acute bacterial

rhinosinusitis and understanding its pathophysiology. The most popular animal models in literature are rabbit, mice and rat[1,2].

Maeyama et al induced chronic sinosinusitis in rabbits by vaccinating Staphylococcus aureus (S. aureus) into the paranasal sinuses in 1981[3]. Bomer et al. presented the first mouse model of acute bacterial rhinosinusitis in 1998 by inoculating mice intranasally with *Streptococcus pneumoniae*[4]. Jacob et al. developed sinusitis in mice with obstruction of middle meatus and bacterial contamination[5]. Rats were first described as sinusitis model in 2005 by Jeon et. al[6]. In 2013 Zhang et al improved fungal rhinosinusitis in rats[7].

There is no report in the literature about developing a rhinosinusitis model in guinea pigs[8-10]. Phillips et. al reported a radiological review that compares the most common rhinosinusitis models in rodents such as rats and mice with guinea pigs, considering anatomy and volume of paranasal sinuses[11]. In this study, the anatomy and volume of the paranasal sinus cavities were defined using microfocal computed tomography (CT) and 2D and 3D images. The paranasal sinuses of mouse were defined similarly to rat sinuses with a decrease in size, while the sinuses of guinea pig were dissimilar in size (Figure 1)[11].

Figure 1. Three-dimensional analysis of rodent paranasal sinus cavities from X-ray computed tomography (CT) scans[11]



Regarding the biggest size of paranasal sinuses in rodents, guinea pig is probably the most suitable model to develop rhinosinusitis[10].

The goal of our study was to determine if the guinea pig which has the biggest size of paranasal sinuses in rodents is a true model to develop acute bacterial rhinosinusitis and to observe the radiological, pathological and microbiological process of inflammation beside three different methods to form acute bacterial rhinosinusitis.

Material and Method

Subjects

900-1000 grams weighing 36 guinea pigs were purchased from the pathogen free animal laboratory of faculty of medicine of this project's co-worker university. Researches obtained the strain and age of the guinea pigs. They would have had minimal exposure to environmental stimuli, including bacterial infections, which have limited immunologic memory. The adaptation time was two weeks before the experiment. Three to five Guinea pigs were in each cage. The animals had free access to food and water. All animal experiments were performed in compliance with a protocol number (2012/78 -ethic committee approval decision no and date) approved by the Institutional Animal Care and Use Committee of Istanbul University in 2012. Intra-muscular doses of 50 mg/kg ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Control %10; Mefar, Istanbul, Turkey) were administered to guinea pigs before all procedures.

Radiological Procedure

36 guinea pigs were divided into four groups, and every week 2 randomly selected guinea pigs were sacrificed and observed microbiologically and histopathologically, which were detected as having rhinosinusitis by computerized tomography (CT) (Figure 2) (Table 1).

Figure 2. Computed tomography scanning.

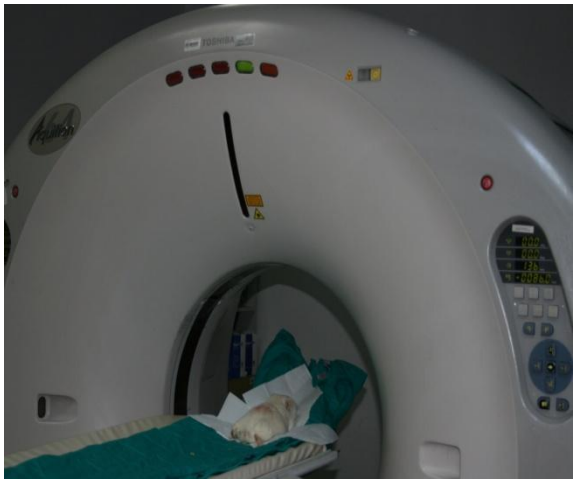


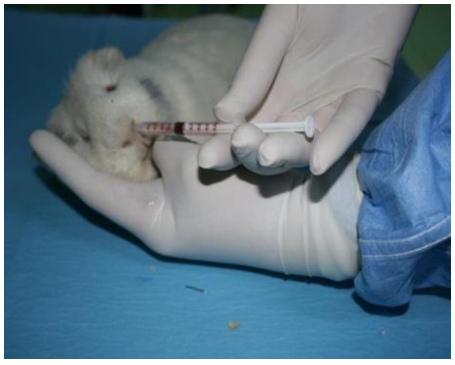
Table 1. Comparison of radiological images between groups (shows number of guinea pigs in each section)

Group 1	**1. Week**	**2. Week**	**3. Week**	**4. Week**
SINUSITIS(+)	4	2	2	-
SINUSITIS(-)	4	4	2	2
Group 2	**1.Week**	**2.Week**	**3.Week**	**4.Week**
SINUSITIS(+)	4	2	2	1
SINUSITIS(-)	5	5	3	2
Group 3	**1.Hafta**	**2.Hafta**	**3.Hafta**	**4.Hafta**
SINUSITIS(+)	9	6	3	1
SINUSITIS(-)	-	1	2	1

Inoculation

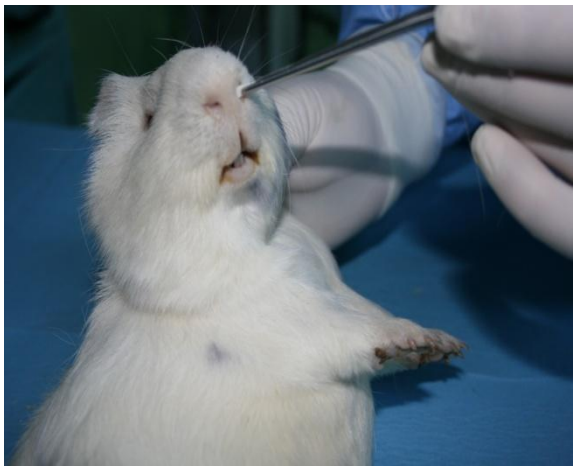
The first group of guinea pigs were inoculated with 0.5 mL Streptococcus pneumoniae (S. pneumoniae) suspension into the right nasal cavities (Figure 3).

Figure 3. Inoculation of guinea pig



Streptococcus pneumoniae suspension was prepared in 1 mL McFarland standards, which contain nearly 10^8 Streptococcus pneumoniae colonies, obtained from Refik Saydam Laboratory (Ankara). In the second group, sponge slivers (0.1x0.3x0.1 cm) were impregnated with sterile saline and were put into the right nasal cavities of guinea pigs. In the third group, sponge strips (0.1x0.3x0.1 cm) were saturated with 0.5 mL streptococcus pneumoniae suspension and were inserted in the right nasal cavities of animals (Figure 4).

Figure 4. Insertion of sponge sliver



The fourth group was control group without any treatment.

Termination

The termination procedure was for 2 guinea pigs from every group per week, especially for guinea pigs that were detected as having rhinosinusitis radiologically. Guinea pigs were killed with a lethal dose of intra-cardiac pentobarbital sodium (120 mg/kg) (Penbital; Bioveta, Ankara, Turkey) (Figure 5, 6).

Figure 5. The right side rhino sinusitis of a guinea pig belongs to group 1 in the first week

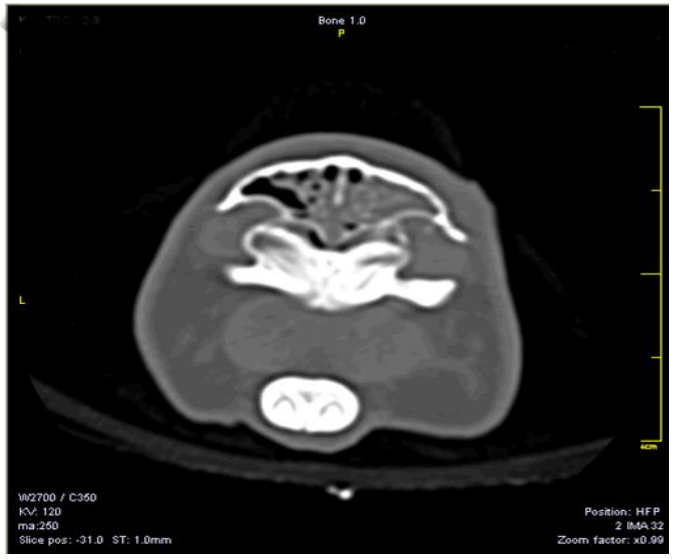
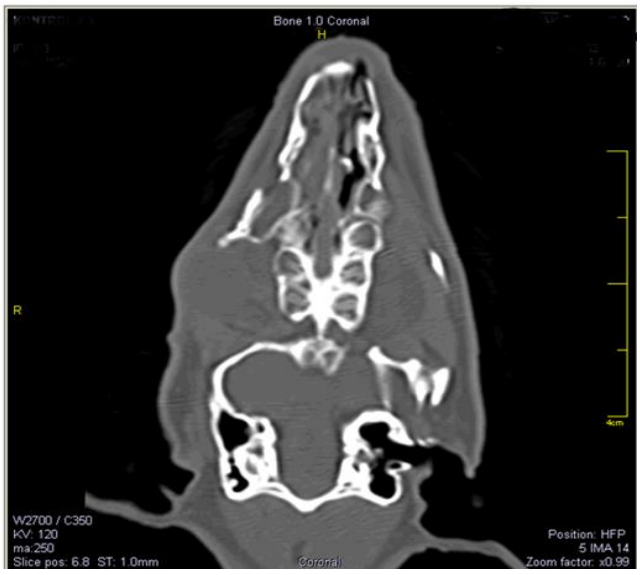


Figure 6. The right side rhino sinusitis of a guinea pig belongs to group 3 in the second week



Nasal Culture

After sacrificing the animals, microbiological samples were taken bilaterally through the nostrils using sterile cotton swabs and placed in Stuart transport medium. Whole culture samples were transferred directly to the microbiology laboratory and studied in aerobic cultures. Aerobic cultures were incubated at 37°C for 24 hours. Isolated bacteria were defined by Standard procedures (Table 2).

Table 2. The comparison between groups of Microbiological distribution

1.Group	1.Week	2.Week	3.Week	4.Week	2.Group	1.Week	2.Week	3.Week	4.Week
S.Pnemoniae	++				S.Pneumoniae				
K.N.S.			+		K.N.S.	+		+	+
Difteroid		+			Difteroid				
S.Aureus					S.Aureus				
A.H.S.		+			A.H.S.				
Gram (-) Basils		+	+	+	Gram (-) Basils	+	+	+	+
3.Group	1.Week	2.Week	3.Week	4.Week	4.Group	1.Week	2.Week	3.Week	4.Week
S.Pneumoniae	+++	++			S.Pneumoniae				
C.N.S.	+		+	+	C.N.S.	+	+	+	+
Difteroid					Difteroid		+	+	+
S.Aureus					S.Aureus	+	+	+	
A.H.S.					A.H.S.		+	+	+
Gram (-) Basils	+	+	+	+	Gram (-) Basils	+	+	+	+

S.pneumoniae -Streptoccus pneumonia; C.N.S. -Coagulase negative staphylococcus; A.H.S.-Alpha hemolytic streptococcus

Histological Examination

The skin of the head was removed. The nasal part of the head was cut off by a coronal incision at 1 mm back of the orbit, and samples lenght of 15 mm were obtained. The mandible and the tongue were removed. The samples were embedded in paraffin, divided at 5 μm thickness and stained with Hematoxylin-Eosin and also were observed with light microscope (Figure 7,8).

Figure 7. Normal mucosa and submucosa (HEX400)

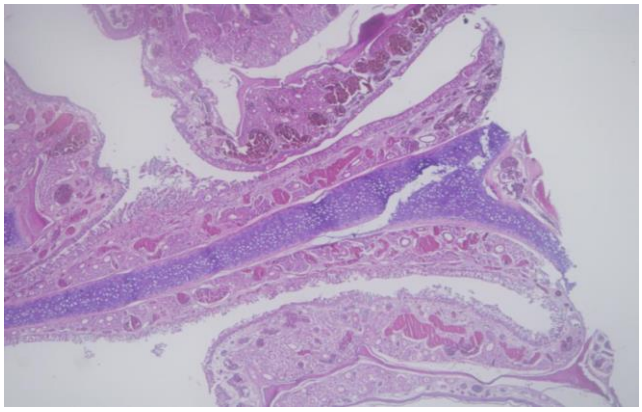
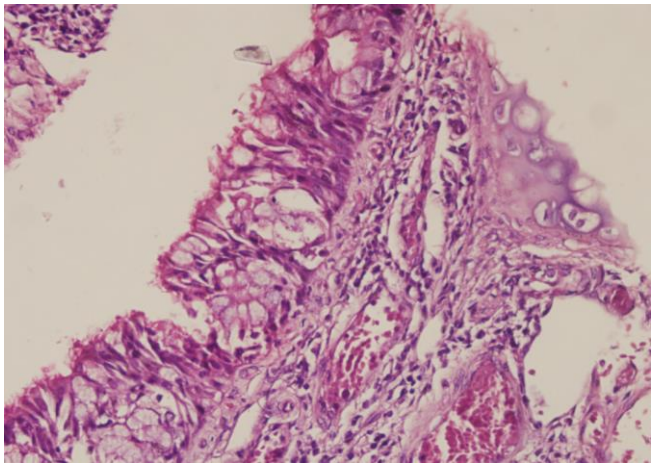


Figure 8. Polymorph nuclear leukocytes (yellow arrow), lymphocyte (arrow)-Dilatation and hemorrhage of blood vessels in area submucosal HEX400



Results

We classified our findings in three sections: radiological, histopatological and microbiological.

Radiological Results

Before the beginning of the study, all computed tomography scans of guinea pigs were acquired, and no air-fluid opacity was dedicated. Every week, computed tomography scans of all guinea pigs were acquired and two guinea pigs that had rhinosinusitis were killed. If sinusitis was not detected, the guinea pig was killed randomly.

After *Streptococcus pneumoniae* inoculation, one guinea pig from the first group died in the first week and excluded from the study. **In the first group,** rhinosinusitis was detected radiologically in 50% of guinea pigs until third week. **In the second group**, rhinosinusitis was detected radiologically in 50% of the guinea pigs, and rhinosinusitis was more severe in group two than group one especially in the third week**.** The severity of the inflammation was evaluated histopathologically. **In the third group,** At the end of the first week, rhinosinusitis was detected radiologically in all guinea pigs (Table 1). In the third week, two guinea pigs which were detected as having rhinosinusitis radiologically, made the suspect of septal perforation. In the third group, rhinosinusitis was detected radiologically in the highest number of guinea pigs and sinusitis were observed in the most severe form. In the fourth week sinusitis detected radiologically. **In the fourth group,** in this group sinusitis was not detected radiologically.

Microbiological Results

In all four weeks, *koagulase-negative stafilococcus, difteroids, stafilococcus aureus (S. aureus), alfa-hemolitic streptococcus, gram-negative basils (Morganella morganii, Asinetobakter, Escherichia coli)* detected, and we think those are the natural flora of guinea pigs.

Histopathological Results

The degeneration of epithelial cells and goblet cell hypertrophy were observed in the histopathological examination of the experimental group. Polymorph cell infiltration in the submucosa was higher in the first two weeks and was particularly severe in the third group. In this case, it refers to an acute inflammation. Symptoms of mononuclear cell infiltration appeared in the third week, and in the fourth week they were at the highest level. Also an increasing tendency in vascular dilatation and hemorrhage was observed.

The most efficient way to develop acute rhinosinusitis is to expose sponge slivers (0,1x0,3x0,1 cm) with Streptococcus pneumoniae suspension into the right nasal cavity of guinea pig. Acute sinusitis has been detected radiologically on the seventh day after inoculation, and resolution has not been seen until the fourth week. In all guinea pigs; sinusitis was detected radiologically and confirmed microbiologically and histopathologically.

Discussion

We designed to develop a rhinosinusitis model on a kind of rodents to overcome the limitations of human studies. Clogging of the sinuses with a foreign material infused with fungi or bacteria was superior to isolated pathogen inoculation and thus confirmed that changes in the nasal fossa and its communication with the paranasal sinuses played a decisive role in the origin of rhinosinusitis. Marks[12] introduced a polyvinyl sponge saturated with pathogenic bacteria (S. Pneumoniae) in the nasal cavity of rabbits; this procedure consists of a combination of macroscopic and bacteriological analyses performed one to ten weeks after the procedure. Signs of inflammation in the sinus mucosa were detected in 83 percent of histological specimens, and

bacterial colonization was observed after one week in more than 50 percent of cases. Our aim was to develop the best model of rhinosinusitis mimicking rhinosinusitis in human with the largest volume of sinuses in rodents (mouse: 0.6, 0.7, and 0.7 mm^3, rat: 8.6, 7.7, 7.0 mm^3, and guinea pig: 63.5, 46.6. mm^3)[11,13].

CT is rarely used in animal studies comparing to clinical human studies. We used CT in our study and detected sinusitis in first week in all groups. In the first week, rhinosinusitis was detected by CT imaging in 50% of guinea pigs of group one, two and was detected in all guinea pigs of the third group. In this study, we confirmed rhinosinusitis microbiologically and histopatologically as well as radiologically. Infection is also confirmed by culturing of organism after inoculation. The number of bacterial colonies grown in group three was higher than group one. Neutrophil clusters in sinus cavities and increasing neutrophils at sinus mucosa were histological proof of acute infection. The microscopical and histopathological changes extended until 30. day. Our results show that we can obviously form a limited model of rhinosinusitis in guinea pigs with *Streptococcus pneumoniae* which is the most common factor of bacterial rhinosinusitis in human.

The uniformity of the infection as shown histologically in all guinea pigs makes this model consistent for rhinosinusitis. Further studies will determine the utilization of this animal model for human disease. Histological characteristics of these models different from each other. Induction of rhinosinusitis with S. aureus is associated with more severe inflammation with exudate in the sinus air spaces[14], whereas toxin- induced rhinosinusitis is characterized by inflammatory cell clusters, hemorrhage with hemolysis in the sinonasal air spaces, and significant loss of epithelial cells[15]. This study showed similar results of acute phase of inflammations as seen in previous studies especially in the third group of guinea pigs[16] like polymorph nuclear leukocyte infiltration, epithelial and squamous cell metaplasia in desquamation. Also bleeding in region of vessels and submucosal epithelial cell degeneration are consistent with the other studies. Goblet cell hyperplasia and subepithelial gland hyperplasia were detected on day 15, which frequently signify the regeneration phase of mucosa membranes of acute inflammation and show the regeneration of acute inflammation or chronic inflammation.

Continuing inflammation is the reason of fibrosis of lamina propria, involution of glands, formation of polyps and bone remodeling. In experimental sinusitis models, nasal cavities or sinus cavities polipoid formation is histologically typical.

In all groups, especially in the third group; concentrated mononuclear cell infiltration was detected in Week 3 and continued in Week 4, which signs chronic inflammation.

All those histopathological findings are the response of the nasal cavity and sinuses to acute or chronic rhinosinusitis. In our study, we found normal flora in guinea pigs and in all groups, especially in the third group we can claim that gram negative bacteria became dominant with the chronic process of sinusitis. Comparing to previous sinusitis models, in our study we didn't

destruct osteums of sinuses directly, sinusitis is formed by three different methods and sinusitis is observed in its natural condition.

Conclusion

We aimed to develop a true model for the natural process of rhinosinusitis by 3 different ways. By radiological and histopathological examinations, we found that the most severe form of rhinosinusitis is created with obstruction and microbiological contamination. It is also believed with this study that guinea pig is a true model in rodents for evaluating rhinosinusitis considering the volume of sinuses beside it is not a perfect model for surgical interventions.

Main Points

Rhinosinusitis can be formed by different ways in animal model.

Guinea pig is a well choice in rodents for rhinosinusitis studies.

Best way to form rhino sinusitis is pathogen inoculated obstruction of nasal passages.

CT is true way for confirmation of rhino sinusitis.

REFERENCES

1. Hilding AH. Experimental sinus surgery; some experiments on ventilation and sinusitis. *Laryngoscope*. 1948;58(10):1098-1102.
2. Kelemen G. The nasal and paranasal cavities of the rabbit in experimental work. *AMA Arch Otolaryngol*. 1955;61(5):497-512.
3. Maeyama T. A study of experimental sinusitis in rabbits. *Auris Nasus Larynx*. 1981;8(2):87-97.
4. Bomer K, Brichta A, Baroody F, Boonlayangoor S, Li X, Naclerio RM. A Mouse model of acute bacterial rhino sinusitis. *Arch Otolaryngol Head Neck Surg*. 1998;124(11):1227-32.
5. Jacob A, Faddis BT, Chole RA. Chronic bacterial rhino sinusitis: Description of a mouse model. *Arch Otolaryngol Head Neck Surg*. 2001;127(6):657-64.
6. Jeon SY, Kim JP, Kim EA, Ahn SK, Kim BG. Rat model of platelet-activating factor-induced rhino sinusitis. *Ann Otol Rhinol Laryngol*. 2005;114(5):393-8.
7. Zhang F, An Y, Li Z, Zhao C. A novel model of invasive fungal rhino sinusitis in rats. *Am J Rhinol Allergy*. 2013;27(5):361-6.
8. Ramadan HH, Meek RB, Dawson GS, Spirou GA, Cuff CF, Berrebi AS. Histologic and immunologic observations of viral-induced rhino sinusitis in the mouse. *Am J Rhinol*. 2002;16(1):61-7.

9. Liang KL, Jiang RS, Wang RC, et al. Upper airway inflammation exacerbates bronchial hyperactivity in mouse models of rhino sinusitis and allergic asthma. *Int Forum Allergy Rhinol*. 2013;3(7):532-42.

10. Drettner B, Johansson P, Kumlien J. Experimental acute sinusitis in rabbit. A study of mucosal blood flow. *Acta Otolaryngol*. 1987;103:432-4.

11. Phillips JE, Ji L, Rivelli MA, Chapman RW, Corboz MR. Three-dimensional analysis of rodent paranasal sinus cavities from X-ray computed tomography (CT) scans. *Can J Vet Res*. 2009;73(3):205-11.

12. Marks SC. Acute sinusitis in the rabbit: A new rhinogenic model. *Laryngoscope*. 1997;107:1579–1585.

13. Dufour X, Kauffmann-Lacroix C, Goujon JM, Grollier G, Rodier MH, Klossek JM. Experimental model of fungal sinusitis: A pilot study in rabbits. *Ann Otol Rhinol Laryngol*. 2005;114:167–172.

14. Kim HS, Jeon SY, Ahn SK, et al. A rat model of acute bacterial rhino sinusitis induced by Staphylococcus aureus. *Korean J Otolaryngol-Head Neck Surg*. 2005;48(6):735-740.

15. Khalmuratova R. A rat model of acute rhino sinusitis induced by alphatoxin of Staphylococcus aureus [dissertation]. Chinju: Gyeong-Sang National University; 2006.

16. Kara CO, Cetin CB, Colakoğlu N, Sengül M, Pakdemirli E. Experimentally induced rhino sinusitis in rabbits. *J Otolaryngol*. 2002;31(5):294-8.