

Serum and Mucosal Nitric Oxide Levels and Efficacy of Sodium Nitroprussid in Experimentally Induced Acute Sinusitis

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Experimental acute sinusitis was induced in 21 New Zealand hybrid rabbits by occluding the ostium and inoculating them with *Streptococcus pneumoniae*. While a group of rabbits with sinusitis was left untreated, two other groups were administered parenteral sodium nitroprussid (SNP) and oral levofloxacin for ten days. While *staphylococci* species, non-hemolytic *streptococcus* and contaminated flora were isolated from the sinuses of controls, *Streptococcus pneumoniae* was re-isolated in two of six untreated rabbits, in one of six SNP administered rabbits and none of the levofloxacin treated rabbits. Serum and maxillary sinus mucosal nitric oxide (NO) levels were correlated. While the mean maxillary sinus NO level of controls was significantly higher than that of untreated rabbits, the mean maxillary sinus and serum NO levels were significantly higher in SNP administered rabbits than in the others. Although goblet cell hyperplasia and squamous cell metaplasia were detected in some slides, edema and neutrophil infiltration were the prominent findings. The most severe inflammatory changes were found in the untreated sinusitis group on the third and fifth days. The earliest improvement was observed in the levofloxacin treated rabbits. It was concluded that NO level is decreased during acute sinusitis and that SNP administration hastens the bacteriological and histological recovery.

Key Words: Levofloxacin, maxillary sinusitis, nitric oxide

INTRODUCTION

In the pathogenesis of sinusitis, ostial dysfunction

is of importance as when the ostium is occluded by mucosal edema, oxygen concentration decreases and in combination with the presence of toxic products in the secretion impairs ciliary functions.¹ It is known that nitric oxide (NO) provides a first-line defense against microorganisms through its antiviral and antimicrobial activity and through its upregulation of ciliary motility.² High NO concentrations were found in paranasal sinuses and it was thought that the lack of NO may contribute to the pathogenesis of sinusitis.^{2,4} In acute sinusitis the most common causative agents are *Streptococcus pneumoniae* and *Hemophilus influenzae*.^{1,5} Empirical and broad-spectrum antibiotics caused the emergence of resistance species.⁶ The aim of this study was to investigate serum and maxillary sinus mucosal NO levels in experimentally induced, acute sinusitis and to document the efficacy of exogenous NO on bacteriological and histological recovery.

MATERIALS AND METHODS

Twenty-one New Zealand hybrid rabbits with a weight range of 2.3-3.4 kg were included in this study. All were allowed *ad libitum* access to food and water during the study. The rabbits were divided into four groups:

Group 1: Three rabbits (controls)

Group 2: Six rabbits with sinusitis without treatment (parenteral saline solution was administered)

Group 3: Six rabbits with sinusitis, which were

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administered parenteral sodium nitroprussid (SNP)

Group 4: Six rabbits with sinusitis, which were administered oral levofloxacin.

In groups 2, 3 and 4, the right nasal cavities were occluded by using an absorbent sponge (Merocel). The next day, 1 ml of type 1 *Streptococcus pneumonia* solution, consisting of 10^7 - 10^9 pneumococci per milliliter, was inoculated into the right maxillary cavities. After 24 hours, 1 ml/kg saline solution and 0.5 mg/kg SNP were injected intravenously to the rabbits of groups 2 and 3, respectively. Levofloxacin 250 mg/d P.O was given to group 4. Drug administration was continued for ten days. On the third, fifth and seventh days, and at the second, third and fourth weeks, one rabbit from each group was decapitated after overdose of intraperitoneal phenobarbital. On the fourth day tamponades were removed. For biochemical investigation, 5 ml blood was obtained intra cardiacally just before decapitation. After the skin of the nasal dorsum above the right maxillary sinuses was incised, the sinus cavities were explored. Bacteriological cultures were taken with cotton swabs and cultivated on blood agar and brain heart agar. Sinus mucosa was removed and prepared for histopathological evaluation and NO measurement. Histopathological specimens were stained with hematoxylin and eosin (H & E) and examined with light microscope.

Sinus mucosa was weighed and exposed with liquid nitrogen and homogenized with 10 ml phosphate tamponade in an ultrasonic dismembrator and filtered. NO was measured by colorimetric method (Boehringer Mannheim).

For statistical analysis, Mann-Whitney-U, ANOVA, and Pearson correlation tests were used. The Ethical Committee of Atatürk University approved the study.

RESULTS

Microbiological results

In controls *staphylococci* species, non-hemolytic *streptococcus* and contaminated flora (*Eschericia coli*, *Moraxella* species, and nonfermentative gram negative bacteria) were isolated. While *Streptococcus pneumonia* was re-isolated from the right sinus on the third and fifth days in group 2, it was re-isolated only on the third day in group 3. In group 4, *Streptococcus pneumonia* was not re-isolated. Isolated microorganisms with respect to the days post-treatment are shown in Table 1.

Biochemical results

Serum and maxillary sinus mucosal NO levels were correlated ($p < 0.01$), and are shown for all 4 groups in Fig. 1 and 2, respectively.

The mean NO level of maxillary sinus mucosa was 4252.3 ± 321.4 nmol/ml, 3790.3 ± 394.3 nmol/ml, 5678.5 ± 980.1 nmol/ml and 4074.8 ± 304.4 nmol/ml in the controls and in groups 2, 3 and 4, respectively. The mean serum NO level was 41.0 ± 1.7 nmol/ml, 33.2 ± 7.4 nmol/ml, 78.5 ± 26.6 nmol/ml and 37.2 ± 5.2 nmol/ml in groups 1, 2, 3 and 4, respectively. Maxillary sinus and serum NO levels were significantly higher in group 3 than in the other groups ($p < 0.05$).

Table 1. Microorganisms Isolated from Maxillary Sinus Mucosa of Groups 2, 3, and 4

	Group 2	Group 3	Group 4
3 rd day	<i>Staphylococcus coagulase</i> (-), <i>Streptococcus pneumonia</i>	<i>E. coli</i> , <i>Streptococcus pneumonia</i>	<i>Staphylococcus coagulase</i> (-)
5 th day	<i>E. coli</i> , <i>Streptococcus pneumonia</i>	<i>Pasteurella multocida</i>	Culture negative
7 th day	Contaminated flora	Culture negative	Culture negative
2 nd week	<i>Staphylococcus coagulase</i> (-)	Contaminated flora	Culture negative
3 rd week	Culture negative	Culture negative	Contaminated flora
4 th week	Contaminated flora	Culture negative	Culture negative

Mucosal NO level of group 1 was significantly higher than that of group 2 ($p < 0.05$).

Histopathological results

Although goblet cell hyperplasia and squamous cell metaplasia were detected in some slides, edema and neutrophil infiltration were the prominent findings. In group 1, edema, congestion and PNL infiltration were not detected. The most severe inflammatory changes were found in group 2, especially on the fifth day (Fig. 3A). These changes began to decrease after the seventh day and disappeared at the fourth week. Changes in group 3 were most severe on the third day, and

then disappeared at the third week. In group 3, congestion was significant during the first 10 days, decreased at the second week and disappeared at the third week. This finding was due to the vasodilator effect of SPN. Histopathological findings of group 3 on the fifth day are shown in Fig. 3B. In group 4, histopathological changes were moderate on the third day and disappeared at the second week.

The earliest improvement was observed in group 4, followed by group 3. The inflammatory findings persisted in group 2. Improvement in group 3 was observed later than in group 4 but earlier than in group 2.

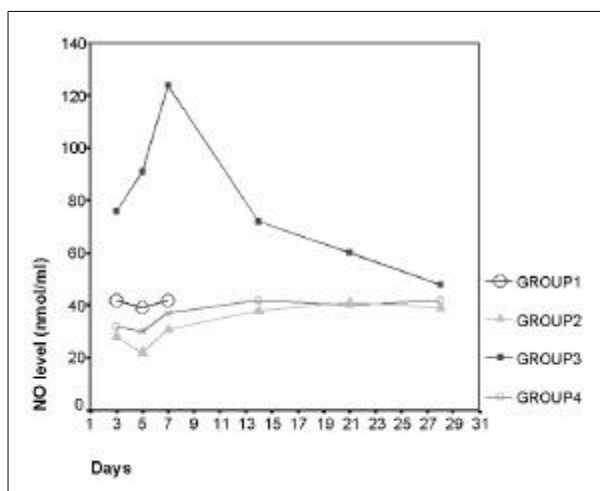


Fig. 1. Serum NO levels in rabbits with respect to days post-treatment.

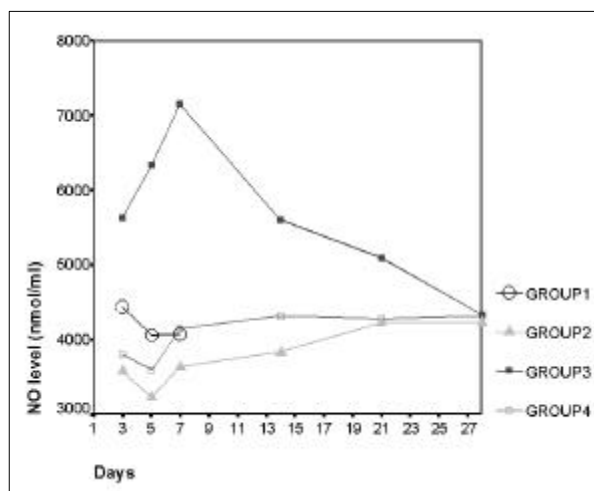


Fig. 2. Maxillary sinus mucosal NO levels in rabbits with respect to days post-treatment.

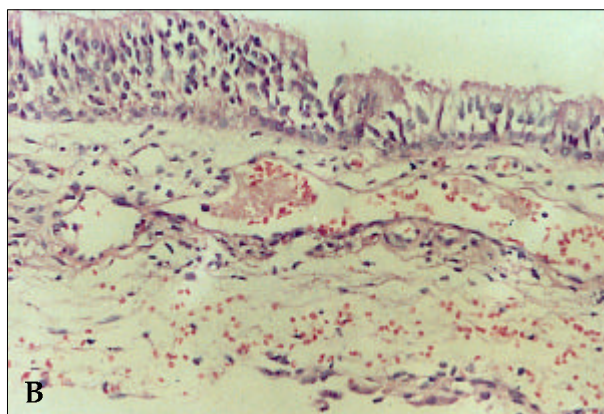
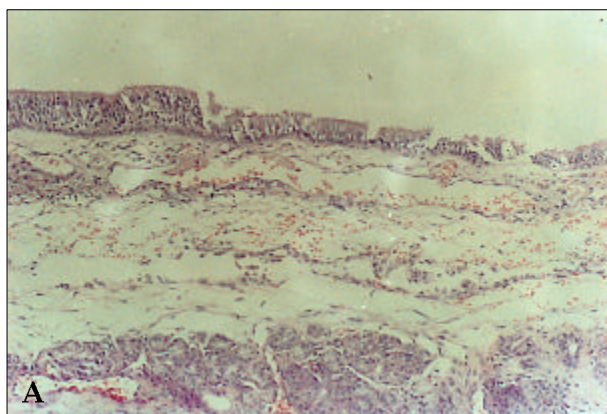


Fig. 3. (A) Submucosal edema and significant neutrophil infiltration were observed on the fifth day in untreated rabbits (H&E $\times 100$). (B) Mild to moderate edema and neutrophil infiltration, and significant vascular congestion were observed on the fifth day in the SNP-administered rabbits (H&E $\times 200$).

DISCUSSION

Mechanical obstruction at the osteomeatal complex plays an important role in the pathogenesis of sinusitis, and obstructing the nasal cavity with a foreign body and then inoculating pathogenic bacteria is a known rhinogenic model of acute sinusitis.⁷ In the present study we used this model for inducing acute sinusitis in rabbits. Since *Streptococcus pneumonia* is the most common agent in acute sinusitis in adults, it was inoculated to the maxillary sinuses of rabbits.

We re-isolated *Streptococcus pneumonia* in two of six untreated rabbits; this ratio was similar to that of some other studies.^{1,7} In the study of Norlander,⁸ investigators did not re-isolate *Streptococcus pneumonia* after seven days but isolated *staphylococcus*, *non hemolytic streptococcus*, *micrococcus* and contaminated flora in more than half of the controls.

In the untreated group, *Streptococcus pneumonia* was not detected after the 5th day probably due to the improvement of ciliary function after removal of the occlusion. In rabbits treated with levofloxacin the causative agent was not re-isolated. It has been demonstrated that levofloxacin has 88% clinical efficacy in acute sinusitis with 10-14 days administration.⁹ In rabbits administered SNP, *Streptococcus pneumonia* was re-isolated only in the third day. SNP is one of the nitrovasodilators that have been used for providing exogenous NO since it is metabolized to NO. In healthy volunteers, Runer et al.,¹⁰ sprayed SNP into the nose and showed that both mucosal blood flow and ciliary beat frequency were increased. Jain et al.¹¹ demonstrated that with NO inhibitor, N-monomethyl-L-arginine-(L-NMMA), a 40% decrease in ciliary movements was observed and that with SNP this effect was reversed. Earlier eradication of *Streptococcus pneumonia* in group 2 compared to group 1 may be due to this effect of NO.

Although it is known that NO concentration is high in paranasal sinuses, recent evidence suggests that the sinuses are not the site of nasal NO production.² In a previous study it was shown that when all the sinus ostia are blocked, nasal NO output is decreased by a mere 12%.¹² In sinusitis, probably due to a decrease in the occlusion of ostium NO, this low concentration affects

bacterial growth.¹³⁻¹⁵ In the rabbits with untreated sinusitis in our study, the NO level was lower than that in the controls.

In untreated rabbits, maxillary sinus mucosal NO value decreased on the third day, re-increased at the second week and returned to normal at the fourth week. In the study of Schlosser et al.,¹⁵ infected sinus lavage of rabbits had elevated levels of NO metabolites that were statistically significant when compared with uninfected sinuses, and NO levels began to return to normal levels during recovery. In rabbits administered levofloxacin, the NO value decreased during the first ten days though it was higher than that of the untreated group. NO was increased after the second week and returned to normal in the fourth week. Serum and maxillary sinus mucosal NO levels were correlated.

In a study performed on children with acute sinusitis, nasal NO level was found to be decreased during illness and increased after antibiotic therapy.¹³

It is known that the rhinogenic model of sinusitis demonstrates typical features of other known models.⁷ In none of the controls were histopathological findings of inflammation observed. In other groups the most severe findings were observed on the third or fifth days after inoculation. In untreated rabbits, histopathological findings were maximum on the fifth day and recovered in the fourth week. In some other studies similar findings were reported.^{7,16,17} The milder histopathological findings observed in SNP-administered rabbits compared to untreated ones and the decrement in the severity of findings earlier than that of untreated rabbits suggest the inhibitor effect of SNP on sinus inflammation.

In conclusion, although present observations suggest that NO level is decreased during acute sinusitis and that SNP administration hastens the bacteriological and histological recovery, further clinical studies are needed to evaluate the therapeutic role of NO donors in acute sinusitis in humans.

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