International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.352.358

Research Article
Effect of Topical Treatment Versus Gavage Feeding of Ciprofloxacin on a Mouse Model of Acute Bacterial Rhinosinusitis

Wei Wang, Xiao-Dong Zhan, Tong Zhou, Xiao-Jing Wang, Hua-Long Qiang and Zhong-Qiang Cheng

Department of Otolaryngology-head and neck surgery, The first Affiliated Hospital of Bengbu Medical College, Zhihuai Road 800, 233003 Bengbu, China

Abstract

Background and Objective: The sinonasal culture studies indicate acute bacterial rhinosinusitis (ABR), an inflammatory condition of nasal and paranasal cavity, characteristic after functional endoscopic sinus surgery. Antimicrobials have been widely used to treat ABR and subside the resultant inflammation. The present investigation compares responses of ciprofloxacin applied topically and by gavage feeding against ABR in mouse model of ABR. **Methodology:** Intranasal inoculation of *Streptococcus pneumoniae* was carried out in C57BL/6 mice for inducing ABR infection. One day after induction, ciprofloxacin administration was done topically and by gavage route in separate animal groups. Animal groups were sacrificed during the course of 5 days after inoculation and treatment. Inflammatory mediators and immunological cells (such as GR1, CD4, CD8 and CD11b cells) were quantified in sinus areas using flow cytometry. Bacterial population was determined by culturing nasal lavage from animals after specified periods. Two-tailed parametric t-test was used for calculation of significant values. **Results:** Ciprofloxacin gavage fed rats showed rapid depletion of microorganisms and of inflammation in contrast to topically treated animals. The reduction in the therapeutic effectiveness of topically applied ciprofloxacin could be due to sub-potent availability, quick nasal depletion or failure of drug suspension to approach the infected site. **Conclusion:** The study concluded that gavage treatment reduced the bacterial count more efficiently against topical treatment of ciprofloxacin in ABR mice animal models.

Key words: Acute bacterial rhinosinusitis, *Streptococcus pneumoniae*, mouse model, topical, gavage

Received: July 10, 2017 Accepted: November 01, 2017 Published: March 15, 2018

Citation: Wei Wang, Xiao-Dong Zhan, Tong Zhou, Xiao-Jing Wang, Hua-Long Qiang and Zhong-Qiang Cheng, 2018. Effect of topical treatment versus gavage feeding of ciprofloxacin on a mouse model of acute bacterial rhinosinusitis. Int. J. Pharmacol., 14: 352-358.

Corresponding Author: Xiao-Dong Zhan, Department of Otolaryngology-head and neck surgery, The first Affiliated Hospital of Bengbu Medical College, Zhihuai Road 800, 233003 Bengbu, China Tel: 0086-552-308635

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute Bacterial Rhinosinusitis (ABR/ABRS) is chiefly characterized by inflammation of border membrane linings of the paranasal sinuses, involving upper breathing region and the sinus[1].

ABRS persists as one of the most notable health issue, known to adversely affect around three million individuals in United States alone, substantiating a major share of health care expenditure. Clinical episodes of ABR, always demand involvement of antibiotics in the therapy. Widespread of ABR, thereby ranks itself as a 5th reason to consumption of antibiotics such a fluoroquinolones, in countries such as US[1].

Streptococcus pneumoniae, marks its presence in the maxillary sinus aspirate in around 60% patients suffering with acute maxillary sinusitis[2]. Antibiotics effectively used against treating ABR, exclusively depends on intensity of infection, combines diseased conditions, history of antibiotic exposure, emergence of antibiotic resistance, sensitivity, acceptability and the treatment cost[3].

Reports convince use of antibiotics topically into the nasal cavity of the patients with ABRS, which is considered as an effective treatment strategy[4-6]. The role of microorganisms is usually doubted to be responsible causing prolonged inflammation in chronic ABR condition. Despite of questions raised against chronic ABR, major issue that pertains for discussion is planning an effective treatment strategy for exacerbations of acute ABR[7]. Research indicated exploration of methods on administration of antibiotics intranasally to animal models, such as rabbit and mouse and quantify inflammatory cell and bacterial cell count. The animal models that could mimic the ABR condition in humans, can be developed post-inoculation by intranasal administration of *Streptococcus pneumoniae* in mouse[8]. The ABR mouse model can serve as measurable object for studying effects of antibiotics, results probably being further extrapolated onto humans[9].

The purpose of the investigation reported hereby was to quantify the effects of ciprofloxacin as a model drug through topical administration versus gavage feeding into ABR induced mouse models using *Streptococcus pneumoniae* inoculation. The study included possible effects on changes in microbial population in cultures of nasal lavage and various cell markers quantified using flow cytometry.

MATERIALS AND METHODS

The studies were carried out in the Pharmacology laboratory and Department of Otolaryngology-head and neck surgery of Bengbu Medical College, China in the month of December, 2016.

Experimental animal: Healthy and uninfected BALB/c mice of either sex were procured from institutional animal department. All the experimental mice were between the age group of 6-8 weeks old. The animals were housed in individual cages maintained within clean and hygienic surroundings, with an access to food and water before they were euthanized. The study was carried out as per the protocols approved by the Institutional Animal Ethical Committee.

Bacteria: Specimen of *S. pneumoniae* (ATCC 49619) was collected from central microbiology laboratory of the institute and was used for developing ABR infection in the experimental animals as reported earlier[7,8]. The strain is known to have antigenic similarity to type 19 *S. pneumoniae*, which has been identified as most common strain present in human maxillary sinus aspirate, developed during ABR infection. The strain was cultivated on solidified agar plates containing blood, into bacterial colonies, which were further subjected to appropriate dilution in sterile saline solution prior to inoculation into mice. The turbidity was maintained at McFarland equivalence value 3, which denotes roughly 1.2×10^9 CFU mL^{-1}. Mice are voluntary nasal breathing animals, hence after placing 25 µL of *S. pneumoniae* suspension in both the nostrils of mice, with inhalation the fluid propagated into the nasal canal.

Bacterial infection: The mice were set aside for 7 days in a clean and hygienic environment so that they can adapt themselves with the surroundings. Mice are voluntary nasal breathing animals, hence after placing 25 µL of *S. pneumoniae* suspension in both the nostrils of mice, the fluid was carried into the nasal path in the course of inhalation. The treatment was initiated 1 day after intranasal administration of the bacterial suspension. Three groups of mice, comprising 10 each, were used for the investigation. Two groups were subjected to treatment with Ciprofloxacin administered topically (Ct group) and Ciprofloxacin administered by gavage feeding (Cg group). The control group included 2 mice which were infected and untreated. The placebo group (P group) was administered with sterile saline solution.

Antibiotic administration: The treatment was commenced 1 day after intranasal inoculation with *S. pneumoniae*. The

ciprofloxacin suspension (0.3% in sterile saline solution) was administered topically to the nasal mucosa into Ct group. In the Cg group of mice, the antibiotic suspension was delivered by gavage feeding (300 mg kg^{-1} in sterile saline solution).

Antibiotic serum level study: Pharmacokinetic study was done by analyzing the serum-ciprofloxacin levels. Blood was derived from orbital sinus of mice after dosing at following time intervals-half, 1, 2 and 4 h (s). The blood collected was subjected to centrifugation at 8000 rpm and resultant serum (10 mL) was inoculated onto sheep agar plates, which were previously inoculated with *S. pneumoniae*. The zone of inhibition was recorded from the plates after 24 h incubation at 37±0.5°C. Standard curve was drawn with the help of known concentration of antibiotic and the extrapolation method was used to determine the experimental values.

Pharmacokinetic parameters that were determined, C_{max}-Maximum concentration of activity, T_{max}-corresponding time of maximum activity and $T_{1/2}$-determined by elimination rate constant by linear regression analysis using T_{max} till the last time point. Area under curve for 24 h (AUC_{0-24}) were calculated using trapezoidal rule by doubling the AUC_{0-12} values.

Nasal cultures: After killing the mice on 3rd and 10th day after inoculation, the nasal lavage was carried out using 200 mL phosphate-buffered saline (PBS). The lavage resultant was subjected to serial dilution (undiluted, 1/10, 1/100, 1/1000 and 1/10,000) and plated on Columbia sheep blood agar plates. After 48 h incubation, the bacterial colonies visible over the plates were counted and the results were indicated as colony-forming units per milliliter (CFU mL^{-1}).

Tissue harvesting and processing: The technique of flow-cytometry (Becton Dickinson verse, San Jose, CA) was performed to determine the presence of cells in the sinuses. After sacrificing the mice from the placebo group, the spleen tissue was used as positive control. Sinuses of individual mouse were exposed by removing the skin and tissues and then bisecting the head through the anteroposterior plane. The tissues were manually separated to expose the sinuses. The resulting harvested tissues were placed in 2 mL PBS containing 2 mg collagenase P (Roche Diagnostics, IN, USA) and incubated at 37±0.5°C on a swirling water bath for 1 h. The degraded tissue suspension was strained through Nytex filter (Sefar-America Inc, NY, USA). The resulting cells were exposed to Dulbecco's minimum essential medium (DMEM) containing 5% fetal calf serum. The cells were subjected to centrifugation at 4°C for 5 min and after discarding the supernatant, the cells were collected as a pellet. Similar process was implemented for all the experiments.

The cell pellet was suspended in 2 mL of the media and further determined using 0.4% trypan blue using a hemocytometer (Sysmex pocH-100i, Sysmex Corporation, Kobe, Japan). The aliquots of live cells placed in each tube were in the range of 1×10^5-5×10^5. The tubes were centrifugation after adding FACS buffer. About 20 mL of 2.4 G2 (an anti FcγRII/III antibody that terminates non-specific binding; procured from BD Biosciences Clontech Labs, Palo Alto, CA, USA) was added in each tube and incubated 15 min at 37±0.5°C. Further incubation was done at 4°C for 45 min after adding 10 mL of antibodies. Next, FACS buffer was added and the composition was centrifuged, concluded finally with addition of 300 µL FACS buffer. The samples were subjected to flow cytometry on a 3-detector BD-FACS can (BD Biosciences Clontech Labs, Palo Alto, CA, USA). The markers examined were neutrophil-GR1, macrophages- CD11b and T cells-CD4 and CD8.

Statistical analysis: The results obtained from flow-cytometric and culture study were normalized statistically. Standard deviations were found to be proportional to the outcome variables. 2-tailed parametric t-tests with values less than 0.05 were considered significant.

RESULTS

In the present investigation, comparison of ciprofloxacin administered topically and gavage feeding was studied in ABR mouse model. On initial day of the study, the mice from each group, except control group, were infected with *S. pneumoniae*. On the 1st day, administration of ciprofloxacin was done topically (Ct group) and by gavage feeding (Cg). The placebo group (P-group) of animals was administered with sterile saline solution. The control group was infected and remained untreated with the antibiotic. Compared to gavage feeding, ciprofloxacin applied topically indicated lesser reduction in the population of bacteria recovered after cultivation. The Cg study group (gavage feeding) indicated significant $p<0.05$ reduction in the bacterial number by 3 and 4 days post infection as illustrated in Fig. 1.

Further the study was targeted to reason why ciprofloxacin was comparatively less effective by topical application. In the initial experiment, responses were recorded after 1 and 3 h of dosing and a significant lowering in the bacterial population was seen in comparison of the control group. The study was supported by analyzing serum levels of

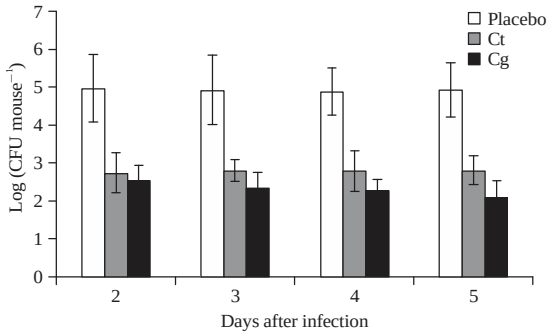

Fig. 1: Bacterial count indicated in mice groups infected with *S. pneumoniae* and their nasal lavage samples were recovered from Ct and Cg group after 2-5 days and cultured

Each bar represents the Mean ± SD (n = 6). In comparison to the placebo (infected but untreated), the bacterial population decreased in both animal groups where ciprofloxacin was administered. Number of bacteria reduced significantly in mouse administered with drug through gavage ($p<0.05$, $p<0.01$)

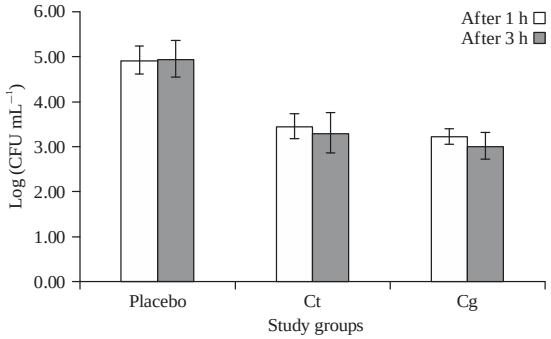

Fig. 2: Population of bacteria in nasal lavage recovered from infected mice on 4th day post infection in Ct, Cg and placebo group. Animal groups were administered with drug and nasal lavage samples were collected after 1 and 3 h of administration

Each bar represents the Mean ± SD (n = 6). Graph indicates reduction in bacterial number in Cg group, followed by Ct group, when compared with placebo group ($p<0.05$)

ciprofloxacin after 1 and 3 h of dosing which was found to be 4.35 and 1.84 μg mL^{-1} in Ct group and 6.79 and 3.19 μg mL^{-1}, respectively as indicated in Fig. 2. This indicated slower absorption and rapid 'washing' of ciprofloxacin through topical delivery.

To elucidate the difference in drug levels subsequent to administration, the animal serum was gathered at predetermined time intervals and analyzed. Pharmacokinetic variables were calculated and are revealed in Table 1. The depletion in ciprofloxacin concentration was found to be rapid after topical administration against gavage delivery-$T_{1/2}$ of 1.2 and 1.4 h, respectively. Ciprofloxacin administered by gavage indicated rapid attainment of C_{max} (T_{max}-0.4 h) in comparison to topical administration (T_{max}-0.7 h). AUC/MIC ratio after 24 h of topical versus gavage administration of ciprofloxacin was found to be 113.4 and 118.6. The serum drug levels after 24 h, indicated 1.2 times higher concentration of ciprofloxacin administered by gavage.

Studies in flow-cytometry indicated an increased granulocytes, macrophages and T-cells levels in infected hosts compared to the control group of animals. In the study group Ct, there was reduction in the levels of GR1 and CD11b, indicating lowering of infection, but the T cells (CD4 and CD8) measure indicated no significant change, represented in Fig. 3a-d. In experimental group Cg, where ciprofloxacin was administered by gavage, undoubtfully the levels of neutrophils and macrophages was lowered and also there was slight reduction in, CD4 and CD8 T-cells. The levels of inflammatory T-cells and macrophages were high in the placebo group.

DISCUSSION

Ciprofloxacin delivered topically in the nasal cavity indicated minimal effect on reduction of bacterial infection compared to the gavage delivery of drug. Local delivery to the nasal mucosa, including direct application, is known to have a greater potential of achieving higher local drug concentration, bypassing first pass effect and rapid onset of action[10,11]. The present investigation involves topical application of ciprofloxacin[12]. The bacterial strain utilized for inducing infection into the experimental mice was sensitive to ciprofloxacin. Literature suggests that in non-neutropenia mice the ratio AUC_{0-24}/MIC should be in between 25-35, for effectiveness of fluoroquinolones against *S. pneumoniae*. The AUC_{0-24}/MIC proportion in this investigation was within the reported range[13-15].

Table 1: Pharmacokinetic variables observed in serum collected from mouse after ciprofloxacin administration

Drug delivery	C_{max} (mg mL^{-1})	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-24} (mg h mL^{-1})	AUC_{0-24}/MIC
Topical application	4.35	1.0	1.2	26.2	113.4
Gavage feeding	6.79	0.6	1.4	30.8	118.6

C_{max}: Maximum concentration indicating activity, T_{max}: Time where maximum concentration is achieved, $T_{1/2}$: Half-life determined using regression analysis by elimination rate constant, AUC_{0-24}: 2 × AUC_{0-12} (Area under the curve plotted between time and concentration for 12 h), MIC: Minimum inhibitory concentration

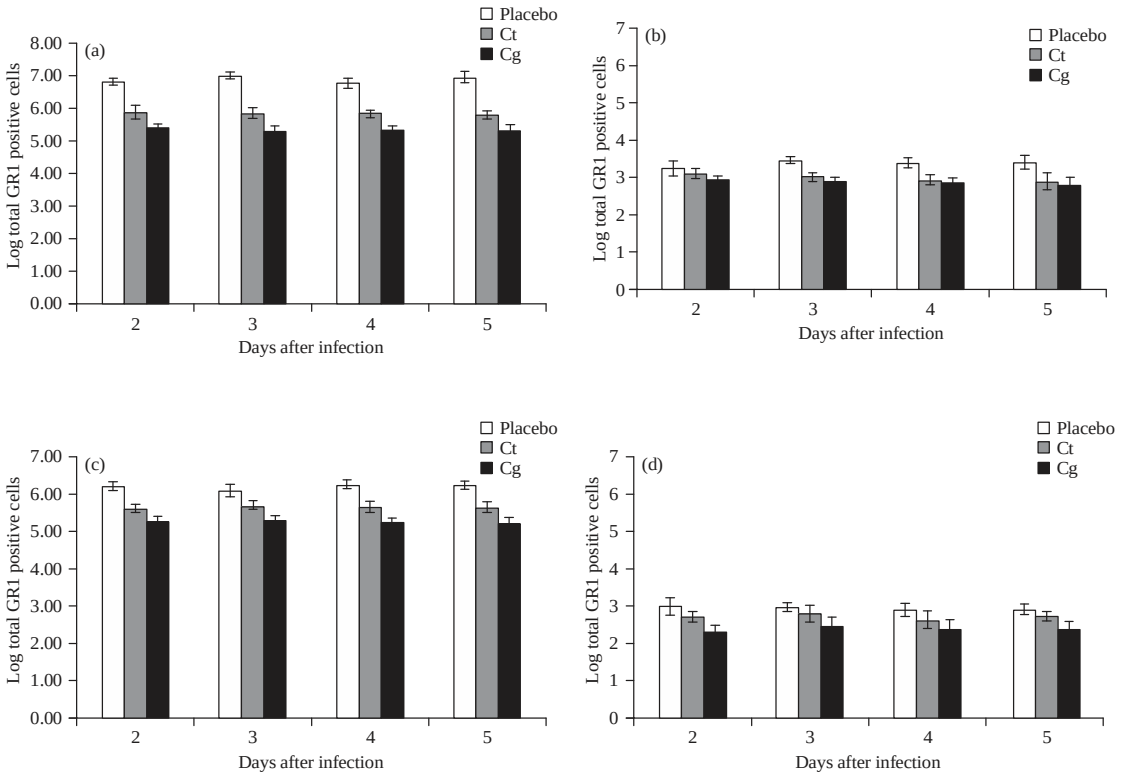

Fig. 3(a-d): Total cell count (GR1, CD11b, CD4 and CD8) after 2, 3, 4 and 5 days of inducing ABR using *S. pneumoniae* and further challenged with ciprofloxacin suspension given topically (Ct) and by gavage (Cg). The placebo group was treated with sterile saline solution. Vertical bars indicate mean cell count and respective error bars indicate standard deviation. There was substantial reduction in levels of GR1 and CD11b after 2-5 days of drug administration ($p = 0.8212$) (a and c). Number of CD4 and CD8 cells indicate significant change, however the number of CD4 cells levels raised after 3 days of infection, whereas Cg group indicated drop in CD4 cells (b). The CD8 cells lowering was significant in Ct group on 4 days, against Cg group, which indicated slight increase in the number of CD8 cells (d)
Each bar represents the Mean±SD

The value of C_{max} was higher in Cg group than in Ct group indicating that the topically applied ciprofloxacin was drained off from the nasal lining, whereas gavage feeding allowed the drug to be in contact with the nasal mucosa. T_{max} value was shorter in gavage feeding group compared to that of topical application. It was concluded that a small difference in the $T_{1/2}$ between Ct and Cg group was due to increase elimination by topical route compared to the gavage feeding. The gavage delivery allowed higher concentration of the drug to diffuse selectively through the nasal membrane. This was further confirmed by calculation of $AUC_{0\text{-}24}$ by trapezoidal rule, showing the higher concentration of drug in the blood.

The nasal lavage of the mice was subjected to culturing onto Columbia sheep blood agar plate for determining the bacterial count after 48 h incubation. The lavage samples were collected 2-5 days after induction of infection from placebo, Ct and Cg group. The CFU mL^{-1} was highest in case of placebo group compared to Ct and Cg group. Amongst the Ct and Cg group of animals, the latter indicated less CFU mL^{-1} value, reason being higher extent of antibiotic residence through gavage feeding than in topical delivery. The topical delivery of ciprofloxacin had higher values of CFU mL^{-1} because of faster elimination from the nasal mucosal barrier. After 1 and 3 h of treatment with ciprofloxacin topically and by gavage, a significant $p<0.05$ reduction the CFU mL^{-1} was found, in comparison with the placebo group.

It has been previously reported that as the bacterial count increases in the sinuses the population of the inflammatory cells increases. Thus, bacterial counts in the lavage can resemble presence of inflammatory mediators in the sinuses[16]. Studies on cell markers from the nasal sinuses collected from the placebo, Ct and Cg group was done using flow-cytometry. The levels of inflammatory cells GR1, CD11b, CD4 and CD8 were found to be n higher in placebo group determined from

2-5 days post inoculation as they were persistently infected without any treatment. There was a substantial drop in the count of GR1 and CD11b cells after administration of ciprofloxacin topically and by gavage, indicating reduction in the inflammation in the sinus. Number of CD4 cells showed slight increase after 3rd day post-infection with topical administration of ciprofloxacin, whereas Cg group indicated reduction in CD4 cells. CD8 cell count was lowered in Ct group on 4 days, compared to the Cg group which indicated slight increase in the C8 count.

Lowered efficiency of the ciprofloxacin administered topically, in comparison to gavage feeding may be attributed to reduced efficacy of the drug to reach the site of inflammation in sufficient concentration due to rapid nasal clearance[17]. Though the study indicated comparative efficiency of ciprofloxacin administration in mouse, but extrapolation onto humans can be tried to get a better insight in treatment strategies of acute bacterial rhinosinusitis. Also, the similar technique may be applied for other drugs proposed for rhinosinusitis treatment.

CONCLUSION

The explored animal model for inducing and studying acute bacterial rhinosinusitis has wide acceptance in terms of specificity of the disease. Two methods for effective administration of ciprofloxacin were evaluated in terms of efficiency and efficacy. After the study it was revealed that, administration of ciprofloxacin was unable to maintain sufficient concentration of the drug due to rapid clearance from the site, contrary gavage delivery was capable of exhibiting higher drug levels thereby had comparable efficiency to treat the ABR. This reduced activity of topically applied ciprofloxacin may be attributed to sub-therapeutic dose levels at the site owing to fast nasal clearance. Whereas, results in animal group treated by gavage delivery ensured that the drug concentrations are optimum to treat the rhinosinusitis triggered by *S. pneumoniae*.

SIGNIFICANCE STATEMENTS

This study discovers the comparative effect of ciprofloxacin, either administered by gavage feeding or topically, to the nasal mucosa for treatment of acute bacterial rhinosinusitis. This study will help the researcher to uncover the critical area of delivering ciprofloxacin and studying its efficiency after administration in mouse model of acute bacterial rhinosinusitis. Thus, a new theory on variable efficiency of ciprofloxacin and similar drugs through topical delivery and gavage feeding in mouse model may be extrapolated on humans.

ACKNOWLEDGMENT

The authors are extremely grateful to Department of Otolaryngology-head and neck surgery, the first Affiliated Hospital of Bengbu Medical College, Bengbu, China for the facilities required to complete the present study.

REFERENCES

1. Anon, J.B., M.R. Jacobs, M.D. Poole, P.G. Ambrose and M.S. Benninger *et al*., 2004. Antimicrobial treatment guidelines for acute bacterial rhinosinusitis. Otolaryngol. Head Neck Surg., 130: 1-45.
2. McCaig, L.F. and J.M. Hughes, 1995. Trends in antimicrobial drug prescribing among office-based physicians in the United States. J. Am. Med. Assoc., 273: 214-219.
3. Marple, B.F., S. Brunton and B.J. Ferguson, 2006. Acute bacterial rhinosinusitis: A review of U.S. treatment guidelines. Otolaryngol. Head Neck Surg., 135: 341-348.
4. Vaughan, W.C. and G. Carvalho, 2002. Use of nebulized antibiotics for acute infections in chronic sinusitis. Otolaryngol. Head Neck Surg., 127: 558-568.
5. Desrosiers, M.Y. and M. Salas-Prato, 2001. Treatment of chronic rhinosinusitis refractory to other treatments with topical antibiotic therapy delivered by means of a large-particle nebulizer: Results of a controlled trial. Otolaryngol. Head Neck Surg., 125: 265-269.
6. Scheinberg, P.A. and A. Otsuji, 2002. Nebulized antibiotics for the treatment of acute exacerbations of chronic rhinosinusitis. Ear Nose Throat J., 81: 648-652.
7. Bomer, K., A. Brichta, F. Baroody, S. Boonlayangoor, X. Li and R.M. Naclerio, 1998. A mouse model of acute bacterial rhinosinusitis. Arch. Otolaryngol. Head Neck Surg., 124: 1227-1232.
8. Blair, C., M. Nelson, K. Thompson, S. Boonlayangoor and L. Haney et al., 2001. Allergic inflammation enhances bacterial sinusitis in mice. J. Allergy Clin. Immunol., 108: 424-429.
9. Wald, E.R., 1998. Microbiology of acute and chronic sinusitis in children and adults. Am. J. Med. Sci., 316: 13-20.
10. Aggarwal, R., A. Cardozo and J.J. Homer, 2004. The assessment of topical nasal drug distribution. Clin. Otolaryngol., 29: 201-205.

11. Leonard, D.W. and W.E. Bolger, 1999. Topical antibiotic therapy for recalcitrant sinusitis. Laryngoscope, 109: 668-670.
12. Miller, T.R., H.R. Muntz, M.E. Gilbert and R.R. Orlandi, 2004. Comparison of topical medication delivery systems after sinus surgery. Laryngoscope, 114: 201-204.
13. Lacy, M.K., W. Lu, X. Xu, P.R. Tessier, D.P. Nicolau, R. Quintiliani and C.H. Nightingale, 1999. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin and ampicillin against *Streptococcus pneumoniae* in an *in vitro* model of infection. Antimicrob. Agents Chemother., 43: 672-677.
14. Lister, P.D. and C.C. Sanders, 2001. Pharmacodynamics of moxifloxacin, levofloxacin and sparfloxacin against *Streptococcus pneumoniae*. J. Antimicrob. Chemother., 47: 811-818.
15. Preston, S.L., G.L. Drusano, A.L. Berman, C.L. Fowler and A.T. Chow *et al*., 1998. Pharmacodynamics of levofloxacin: A new paradigm for early clinical trials. J. Am. Med. Assoc., 279: 125-129.
16. Blair, C., R.M. Naclerio, X. Yu, K. Thompson and A. Sperling, 2005. Role of type 1 T helper cells in the resolution of acute *Streptococcus pneumoniae* sinusitis: A mouse model. J. Infect. Dis., 192: 1237-1244.
17. Sahin-Yilmaz, A., F. Baroody, A. Markaryan, K. Thompson, G.M. Wall and R. Naclerio, 2008. Effect of topical ciprofloxacin/dexamethasone or dexamethasone alone on acute *Streptococcus pneumoniae* rhinosinusitis in mice. Otolaryngol. Head Neck Surg., 138: 340-346.