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The effect of treatment with moxifloxacin or azithromycin on acute bacterial rhinosinusitis in mice

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Summary
Objective: Acute bacterial rhinosinusitis, which is a major health problem, is treated with antibiotics. We developed a mouse model of acute bacterial rhinosinusitis to gain a better understanding of the pathophysiology of the disease. Our goal was to investigate the response to acute rhinosinusitis when treated with either a bactericidal or a bacteriostatic antibiotic.
Methods: C57BL/6 mice were infected intranasally with *Streptococcus pneumoniae*. One day after inoculation, the mice were treated with either moxifloxacin (bactericidal) or azithromycin (bacteriostatic). Different groups were euthanized during the first five days post-inoculation. Bacterial counts from nasal lavage culture and the cell markers GR1, CD11b, CD3, CD4, and CD8 in sinus tissue were evaluated by flow cytometry.
Results: Azithromycin led to rapid clearance of the bacteria and of the inflammation in contrast to placebo. Surprisingly, moxifloxacin showed a limited effect. Investigations of this limited effect of moxifloxacin suggested a high metabolic clearance, a low concentration at the site of infection, and low persistent post-antibiotic effects of moxifloxacin in mice.
Conclusion: Our animal model of acute sinusitis has great utility for studying the disease, but the difference between mice and man must always be considered in making extrapolations from animal experiments to the human experience.

Introduction

Acute sinusitis, an inflammation of the lining membrane of any of the paranasal sinuses, affects millions of people. Acute sinusitis frequently follows a viral upper respiratory tract infection and involves the maxillary sinus. Thus, if the average American has four viral colds per year, and 1% of

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colds lead to acute sinusitis, then there are about 10 million episodes of acute sinusitis in the USA each year.[1] In approximately 60% of patients with acute maxillary sinusitis, bacteria can be isolated from a maxillary sinus aspirate, the most common organism being *Streptococcus pneumoniae*. Episodes of acute bacterial rhinosinusitis are usually treated with antibiotics. Thus, each year, millions of prescriptions are written for the treatment of acute bacterial rhinosinusitis.[2] Despite the magnitude of the problem, many questions remain as to how best to treat an acute episode.

Antimicrobial drugs have been classified on the basis of their action in vitro as being either bactericidal or bacteriostatic. For bactericidal drugs, the minimal bactericidal (MBC) or lethal concentration is close to or identical to the minimal inhibitory concentration (MIC). For bacteriostatic drugs, little if any killing occurs at the MIC; only bacteriostasis occurs, which is reversed on removal of the chemotherapeutic agent. This distinction is blurred in vivo because bacteria whose multiplication has been arrested by bacteriostatic drugs can be killed by the cellular and humoral immune defenses of the host. Moxifloxacin, an 8-methoxy quinolone, exerts a bactericidal effect on *S. pneumoniae* by inhibiting the type II topoisomerases, DNA gyrase and topoisomerase IV,[3] whereas azithromycin, a 15-membered-ring macrolide, inhibits bacterial protein synthesis by binding to two sites on the bacterial 50 S ribosome (domains II and V), which causes dissociation of transfer RNA and termination of peptide linking. Macrolides are classified as bacteriostatic for most Gram-positive bacteria, in particular streptococci.

We previously developed a mouse model of acute bacterial rhinosinusitis after intranasal inoculation with *S. pneumoniae*[4,5] that mimics the disease in man.[4,6] Our objective in this study was to determine whether treatment of acute bacterial rhinosinusitis caused by *S. pneumoniae* with a bactericidal drug (moxifloxacin) compared to a bacteriostatic drug (azithromycin) caused less infection and more rapid resolution of the inflammation.

Materials and methods

Experimental animals

Pathogen-free 6- to 8-week-old C57BL/6 mice of either sex were purchased from Jackson Laboratories (Bar Harbor, ME, USA). The animals were kept in microisolator cages in a pathogen-free environment. Each group of animals was kept isolated from the other groups in a biohazard containment facility. All animals were used in protocols approved by the Animal Care and Use Committee of the University of Chicago.

Antibiotic administration

Antibiotics were administered via 200 μL gavage. The doses of moxifloxacin and azithromycin were 100 mg/kg and 200 mg/kg, respectively. The control group was administered the sterile water diluent alone. The regimens were given twice daily or four times daily, depending on the experiment.

Antibiotic serum levels

The pharmacokinetics of moxifloxacin (Bayer Corporation, West Haven, CT, USA) and of azithromycin (Pfizer Laboratories, Groton, CT, USA) were tested in the serum of mice. Blood was drawn from the orbital sinus of mice at 0.5, 1, 2, and 4 h after antibiotic dosing. The blood was centrifuged, and the serum was collected. Ten microliters of serum were then aliquoted onto sheep blood agar plates coated with *S. pneumoniae*. The plates were incubated for 24 h at 37 °C, after which zones of inhibition were measured. A standard curve was formed by use of the known concentrations of the antibiotics. Quantitative values were determined by extrapolation from the standard curve.

Pharmacokinetic analyses were performed. Parameters were defined as follows: C_{max} was the maximal concentration or activity observed; T_{max} corresponded to the time that the peak was observed; $T_{1/2}$, or half-life, was determined from the elimination rate constant as calculated by linear regression analysis from T_{max} to the last time point. Area under the time–concentration curves (AUCs) over 12 h (AUC_{0-12}) were calculated by use of the trapezoidal rule; AUC_{0-24} was defined as twice the AUC_{0-12}. The 24-h AUC/MIC ratios were calculated by dividing the AUC_{0-24} by the MIC for the specific drug.

Infection

S. pneumoniae (ATCC 49619) was used for induction of acute sinusitis, as described previously.[4,5] The strain used is antigenically similar to type 19 *S. pneumoniae*, the most common strain cultured from human sinuses.[4] The MICs of azithromycin and moxifloxacin for this strain were 0.25 μg/mL.

S. pneumoniae was grown on blood agar plates, and colonies were suspended in sterile saline solution immediately before inoculation of the mice. A turbidity equivalent to McFarland no. 3 was used, which corresponds to approximately 1.2×10^9 colony-forming units per milliliter. Twenty-five microliters of suspension *S. pneumoniae* were placed in each nostril. Because mice are obligatory nasal breathers, the fluid was drawn into the nasal passage during inhalation.

Nasal cultures

Mice were sedated and nasal lavage was performed with 200 μL of phosphate buffered saline (PBS). The lavage liquid was then serially diluted (neat, 1:10, 1:100, 1:1000, and 1:10 000) and, at each dilution, plated onto Columbia sheep blood agar plates. The plates were incubated for 48 h and then counted. The results were quantified as colony-forming units per milliliter (CFU/mL).

Tissue harvesting and processing

Flow-cytometric analysis was used for quantifying cells present in the sinuses. The mice were euthanized, and the spleen was harvested as a positive control. We removed the skin and tissue from the head and then sagittally bisected the skull, exposing the sinuses. The tissue from the sinuses was removed manually. The harvested tissue was placed in 2 mL of PBS. We added 2 mL of PBS to 2 mg/mL collagenase P (Roche Diagnostics, IN, USA) to give a final concentration of 1 mg/mL. The tissue was incubated at 37 °C for 1 h in a water bath shaker. After tissue degradation, the suspension was passed through a Nytex filter (Sefar-America Inc, NY, USA) and the cells recovered in Dulbecco's minimum essential

medium (DMEM) with 5% fetal calf serum (medium). Next, we centrifuged the cells for 5 min at 4 °C, 1350 rpm, and discarded the supernatant (centrifuge conditions were the same for all experiments). We then resuspended the cell pellet in 2 mL of medium and quantified cells by use of 0.4% trypan blue on a hemocytometer. Using the calculated live cell number, we aliquoted cells to a concentration between 1 and 5×10^5 per FACS tube. All of the tubes were filled with FACS buffer and centrifuged. We then added 20 μL of 2.4 G2 (an anti-FcγRII/III antibody that stops nonspecific binding; obtained from BD Biosciences Clontech Labs, Palo Alto, CA, USA), and incubated the tube for 15 min at room temperature. Ten microliters of the antibody or antibodies diluted to the titration amount were added and incubated for 45 min at 4 °C. After incubation, we again added FACS buffer and centrifuged. Finally, we added 300 μL of FACS buffer. Flow cytometry was performed on a 3-detector BD FACScan or a 6-detector BD LSR. The markers we examined were GR1 (neutrophils), CD11b (macrophages), CD3, CD4, and CD8 (T cells).

Antibiotic susceptibility test

In order to ensure that *S. pneumoniae* was susceptible to moxifloxacin, we sampled two broth solutions containing *S. pneumoniae*. One solution contained 1 μg/mL of moxifloxacin, and the other served as a control. The broth solutions were sampled at 0, 1, 2, 4, and 8 h. The samples were plated on agar plates for 24 h at 37 °C. The CFUs were then counted and the numbers of bacteria quantified.

Statistical analysis

Log conversion of the flow-cytometric and culture data was performed for normalizing the data. Parametric two-tailed *t*-tests were used. A p value <0.05 was considered to indicate significance.

Results

We first compared treatment with moxifloxacin, azithromycin, and placebo. On day 0, we infected mice with *S. pneumoniae,* and on day 1, we began administering the treatments twice daily. On days 2—5, we euthanized mice from each group. The control mice became infected and remained infected through five days of observation. Treatment with azithromycin reduced the amount of infection within 24 hours of administration. By days 4 and 5 post-infection, the bacteria were essentially eliminated. The surprising result was a lack of effect of moxifloxacin on the number of bacteria recovered (Figure 1).

We next performed experiments to determine why moxifloxacin was ineffective in our model. In the first experiment, we evaluated the response of the mice at 1 h and 3 h post-dosing. In this experiment, we evaluated the response at day 4 post-infection, looking at the time between the last doses of moxifloxacin and harvesting of the tissue. Both timing intervals showed a significant reduction in the bacterial counts compared to control (Figure 2), but the results were not equivalent to the effect of azithromycin. We also gathered serum to measure drug levels. Moxifloxacin serum concentrations 1 h and 3 h post-dosing were 4.8 μg/mL and

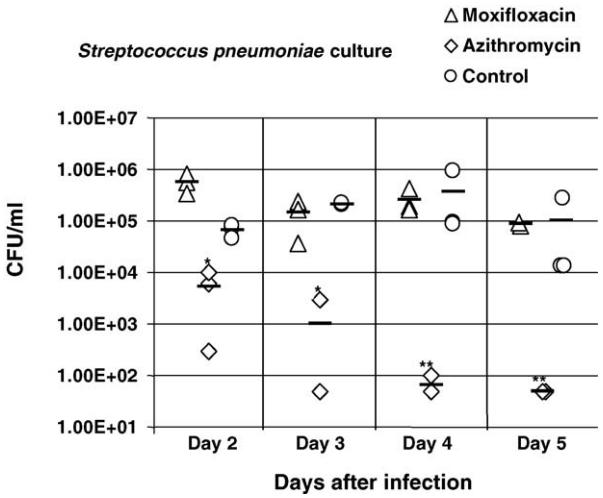

Figure 1 Numbers of bacteria recovered from nasal lavage culture 2—5 days after infection with *S. pneumoniae*. Mice were treated with 12-hourly doses of 100 mg/kg moxifloxacin, 200 mg/kg azithromycin, or sterile water (control). Horizontal bar represents mean value. The numbers of bacteria in the azithromycin-treated mice decreased significantly in comparison with the moxifloxacin-treated or control groups ($^{*}p < 0.05$, $^{**}p < 0.01$).

1.95 μg/mL, respectively. This demonstrated a rapid metabolism of moxifloxacin in the mice.

To understand this rapid metabolism further, we measured the serum levels after dosing. The serum from three mice was collected at 0.5, 1, 2, and 4 h after a single gavage dose of 100 mg/kg moxifloxacin or 200 mg/kg azithromycin. Pharmacokinetic parameters for moxifloxacin and azithromycin are shown in Table 1. The elimination of moxifloxacin was more rapid than azithromycin with a $T_{1/2}$ of 1.4 h. Both azithromycin

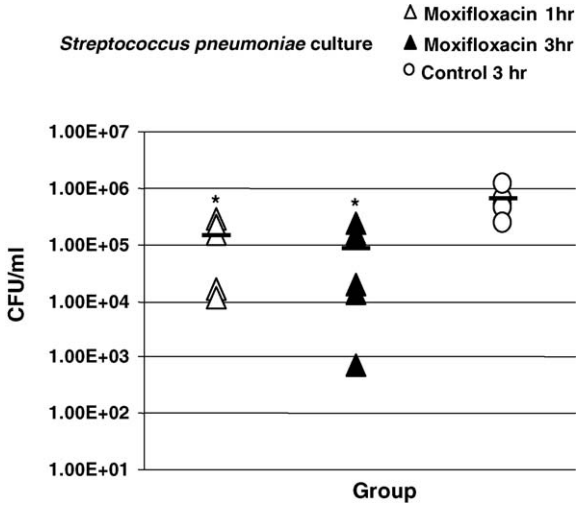

Figure 2 Bacterial counts in nasal lavage culture at day 4 post-infection in mice treated with 12-hourly doses of 100 mg/kg moxifloxacin and sterile water (control). Samples were collected 1 h and 3 h post-dosing of moxifloxacin and 3 h post-dosing of sterile water. Horizontal bar represents mean value. There was a significant decrease in the numbers of bacterial counts in mice treated with moxifloxacin 1 h and 3 h post-dosing compared with the control group ($^{*}p < 0.05$).

Table 1 Pharmacokinetic parameters of moxifloxacin and azithromycin in mouse serum

Drug	C_{max} (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-24} (μg h/mL)	AUC_{0-24}/MIC
Moxifloxacin	4.8	1	1.4	29.9	119.7
Azithromycin	8.9	0.5	1.6	50.4	201.6

C_{max}, maximal concentration or activity observed; T_{max}, time that the peak was observed; $T_{1/2}$, half-life, was determined from the elimination rate constant as calculated by linear regression analysis from T_{max} to the last time point; AUC_{0-24}, twice the AUC_{0-12} (area under the time—concentration curves over 12 h); MIC, minimal inhibitory concentration.

and moxifloxacin displayed a short lag in absorption before reaching T_{max}, with azithromycin attaining a peak concentration earlier than did moxifloxacin. The 24-h AUC/MIC ratios of azithromycin and moxifloxacin were 201.6 and 119.7, respectively. The serum AUC_{0-24} of azithromycin was 1.7-fold higher than that of moxifloxacin. We also tested the susceptibility of *S. pneumoniae* to moxifloxacin. The bacteria are sensitive to 1 μg/mL moxifloxacin (four times the MIC), but it takes about 4 h to produce effective killing (Figure 3).

Finally, we increased the dosing frequency of moxifloxacin from 100-mg/kg twice daily to 100-mg/kg 6-hourly to try to counter its high metabolic rate in mice. Azithromycin or placebo was dosed as in the first experiment. We increased the number of mice to seven in each group in order to increase the statistical power. Moxifloxacin significantly decreased the number of bacteria in the sinuses when compared to the placebo control, although not to the level of azithromycin (Figure 4).

Flow-cytometric analysis showed that there was an increase in granulocytes, macrophages, and CD3-, CD4-, and CD8-positive T cells in all infected sinusitis groups compared to an uninfected group (wild type). Among the infected groups, the granulocytes and macrophages (GR1) in the azithromycin-treated group had a tendency to decrease when the infection subsided, whereas the levels of CD3-, CD4-, and CD8-positive T cells were still high. In contrast, all inflammatory cells were high in the persistently infected groups. Interestingly, the inflammatory cells and CD3-, CD4-, and CD8-positive T cells in the moxifloxacin-treated group were at significantly higher levels than in the untreated control group (Figure 5).

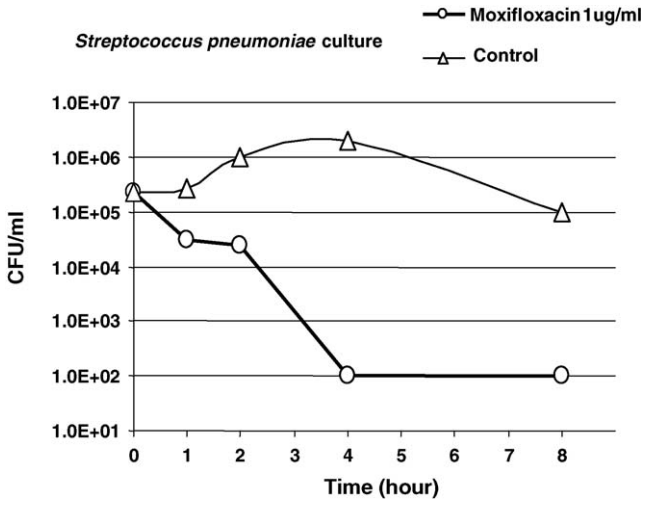

Figure 3 Antibiotic susceptibility test demonstrated that 1 μg/mL moxifloxacin was effective in killing *S. pneumoniae*.

Discussion

In man, both moxifloxacin and azithromycin are effective agents in the treatment of acute bacterial rhinosinusitis caused by susceptible strains of *S. pneumoniae*.[7–18] In our study, azithromycin significantly reduced the bacterial counts in the sinus compared with placebo treatment. The reduction in the bacteria recovered was accompanied by faster resolution of the inflammation associated with the infection. These results parallel the effect in man.

Surprisingly, moxifloxacin showed a minimal effect on infection. We first eliminated the possibility that the strain of *S. pneumoniae* studied was resistant to this antibiotic. We next looked at the 24-h AUC/MIC and the peak/MIC ratios, the primary pharmacokinetic/pharmacodynamic (PK/PD) parameters used to determine the efficacy of the fluoroquinolones in man. Animal survival studies in mice without neutropenia, as well as clinical trials, all suggest that the magnitude of the 24-h AUC/MIC ratio required for the efficacy of fluoroquinolones or azithromycin against *S. pneumoniae* is in the 25—35 range.[19–21] The AUC/MIC ratios for moxifloxacin and azithromycin in our experiments were more than 35, which is consistent with published reports. The fact

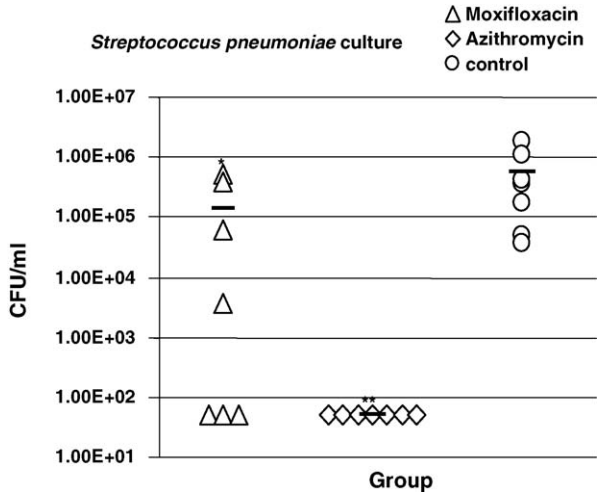

Figure 4 Bacterial counts from nasal lavage culture on day 5 post-infection in mice treated with 6-hourly doses of 100 mg/kg moxifloxacin, 12-hourly doses of 200 mg/kg azithromycin, and sterile water (control). Horizontal bar represents mean value. The results showed that the bacterial numbers in the moxifloxacin-treated mice decreased significantly in comparison with the control group ($^{*}p < 0.05$), and in the azithromycin-treated mice decreased significantly in comparison to both the moxifloxacin-treated and control group ($^{**}p < 0.01$).

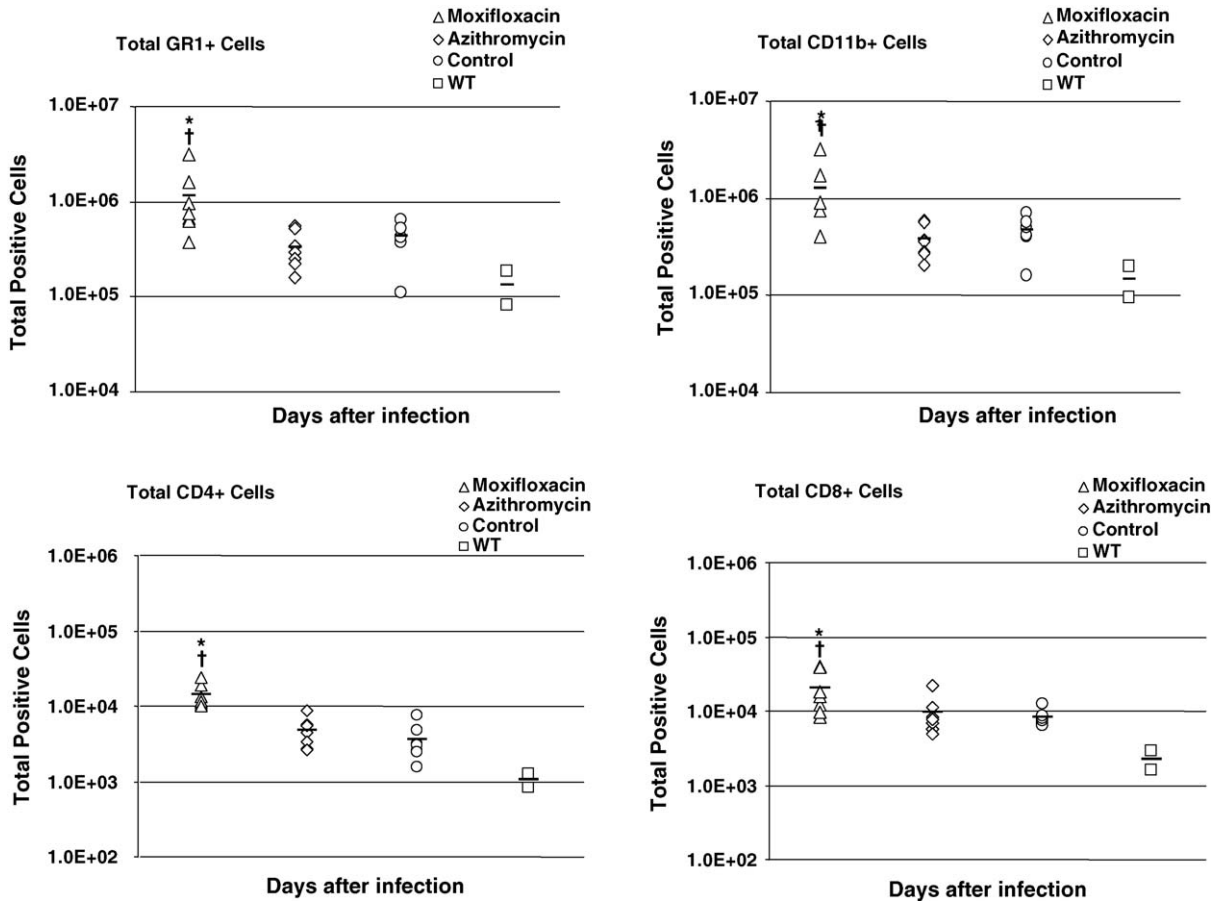

Figure 5 Numbers of total cells stained with GR1, CD11b, CD4, and CD8 at day 5 post-infection from sinus tissue of mice infected with *S. pneumoniae* and treated with 6-hourly doses of 100 mg/kg moxifloxacin, 12-hourly doses of 200 mg/kg azithromycin, and sterile water (control). Horizontal bar represents mean value. There was a significant increase in the numbers of GR1-, CD11b-, CD4-, and CD8-stained cells in the moxifloxacin-treated mice in comparison with the azithromycin-treated ($^{*}p < 0.05$) or control group ($^{\dagger}p < 0.05$). Wild type (WT) mice received no treatment or infection.

that the PK/PD goals were met, and bacterial clearance did not occur, may suggest that moxifloxacin levels at the site of infection are lower than in serum.

Siefert et al.,[22] however, demonstrated high concentrations of moxifloxacin in bile ducts and urine at 5 min and 1 h after IV administration, indicating a rapid onset of excretion in mice. The serum half-life of moxifloxacin in mice was about 1—2 h,[22—25] whereas azithromycin showed an extended serum half-life of about 6 h in mice.[17,18] Our results agree with these findings and were further supported by the observation that, after we increased the frequency of dosing of moxifloxacin, the outcome improved.

To explain the reduced effect of moxifloxacin, we hypothesized that, because of the high metabolism in mice, the drug may have killed some of the bacteria, but then, because it was rapidly metabolized, the *S. pneumoniae* repopulated the sinus (*S. pneumoniae* doubled every 20 minutes).

Four hours post-dosing, the moxifloxacin serum level was 1.1 μg/mL, which was above the MIC of 0.25 μg/mL. It should have a bactericidal effect at this level. This notion was demonstrated by a serum inhibition assay in vitro, but it took about 4 h to induce killing of the bacteria. The bacterial counts in the sinuses 3 h post-dosing in vivo suggest that the killing effect was not as great as in vitro. It is possible that moxifloxacin levels were lower in the mouse sinus mucosa and extracellular fluid than in the serum. This would contrast with the higher concentrations of moxifloxacin found in human sinus tissues compared to the blood.[22,26] Another potential explanation for our observations is that the killing effect of moxifloxacin is concentration-dependent[10,11,27,28] and thus, due to high metabolic clearance, moxifloxacin had a limited effect in our mouse model.

A further pharmacodynamic property exhibited by both moxifloxacin and azithromycin is continued suppression of an organism's growth after antimicrobial exposure,[27—29] known as the post-antibiotic effect (PAE). The PAEs for fluoroquinolones for both Gram-positive and Gram-negative isolates are generally in the range of 1.5—2.5 h. Post-antibiotic subinhibitory effects and post-antibiotic leukocyte enhancement have also been described. These effects were not sufficient to influence bacterial clearance in our model. Azithromycin has a longer duration of subinhibitory concentrations, which may have contributed to its PAEs and hence efficacy.[30]

Although we could not address our initial question about comparing a bacteriostatic with a bactericidal drug, this study shows the importance of the antimicrobial concentrations at the site of infection as well as of the pharmacokinetics and pharmacodynamics of antimicrobial drugs. It also

suggests that pharmacodynamic principles may not always apply across species. Finally, our mouse model proved helpful in the exploration of the mechanisms of antimicrobials in the treatment of acute bacterial rhinosinusitis.

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Conflict of interest: No conflict of interest to declare.

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