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Bactrim reduces the inflammatory response in a murine model of acute rhinosinusitis*

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SUMMARY

Objective: *To determine whether treatment with an antibiotic (trimethoprim-sulfamethoxazole) reduced the inflammatory response in a murine form of Streptococcus pneumoniae-induced rhinosinusitis.* ***Design:*** *We randomized 18 C57BL/6 mice to either treatment with intraperitoneal trimethoprim-sulfamethoxazole (Bactrim, 30 mg/kg) or no treatment (control). After 2 days, we inoculated all C57BL/6 mice intranasally with a Bactrim-susceptible strain of Streptococcus pneumoniae, ATCC 49619, suspended in Trypticase soy broth. At day 5 after bacterial inoculation, we sacrificed the mice and prepared histopathologic sections of their sinuses after culturing their nasal cavities by lavage.* ***Setting:*** *Animal care facility at a tertiary, academic institution.* ***Methods:*** *The histopathologic sections of the sinuses were examined in a blind manner for the percent of sinus cavity area occupied by neutrophil clusters, and for the number of neutrophils per square millimeter of sinus mucosa.* ***Results:*** *The Bactrim group had a significantly smaller sinus area occupied by neutrophil clusters (1.58%±1.13 vs 4.38%±3.41; $P<0.05$), significantly fewer neutrophils infiltrating the mucosa (58.81±29.63/mm^2 vs 105.85±48.49/mm^2; $P<0.05$), and significantly less growth of Streptococcus pneumoniae colonies in the intranasal cultures (8 few and 1 moderate vs 3 few, 3 moderate, and 1 many; $P=0.05$) compared to the control group.* ***Conclusion:*** *In our murine model of acute rhinosinusitis, Bactrim decreased the number of neutrophil clusters in the sinus cavities, the number of neutrophils infiltrating the sinus mucosa, and the growth of Streptococcus pneumoniae. We propose that our murine model can be used for the study of the pathophysiology and treatment of acute rhinosinusitis.*

Key words: bactrim, mouse, sinusitis, streptococcus pneumoniae

INTRODUCTION

Streptococcus (S.) pneumoniae is the most frequently isolated bacterial pathogen in episodes of acute sinusitis (Evans FO Jr et al., 1975), and physicians commonly use antibiotics that are active against this pathogen to treat the disease (McCaig LF, Hughes JM, 1995; Axelsson A et al., 1973). Trimethoprim-sulfamethoxazole (Bactrim), one of the antibiotics to which many strains of *S. pneumoniae* are susceptible, has been used in the treatment of acute sinusitis for many years (Hamory BH et al., 1979). Studies have demonstrated that appropriate treatment with antibiotics can decrease the high titer of cultured bacteria from maxillary sinus aspirates of patients with acute sinusitis (Hamory BH et al., 1979). More recently, however, studies of community-acquired sinusitis suggest no difference in the clinical course of acute sinusitis treated with placebo or with amoxicillin (Dohlman AW et al., 1993; van Buchem FL et al., 1997). Although Bactrim and other antibiotics are believed to treat the infection effectively, the histopathologic changes associated with acute sinusitis and the effect of treatment have not been described.

A rabbit model is available for the study of sinusitis in animals, and ostial obstruction is a prerequisite for creating a sinus infection (Bolger WE et al., 1997; Bolger WE et al., 1997; Marks SC 1997; Marks SC 1998). We recently developed a murine model because it offers a greater potential to study genetic susceptibility to infection and inflammation, even though surgical mani-

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pulation is limited (Bomer K et al., 1998). In this study, we examined the effect of pretreatment with Bactrim on the histopathologic and bacteriologic outcome of acute sinusitis in our murine model.

MATERIALS AND METHODS

Mice

C57BL/6 female mice 4 to 6 weeks of age, weighing 16 to 19 grams, were purchased pathogen-free from Jackson Laboratory and kept in the Carlson Biocontainment Suite Facility at the University of Chicago. All protocols were approved by the Institution of Animal care and use Committee of the University of Chicago. All manipulations of the animals before sacrifice were conducted in a class II biosafety hood with adherence to strict biosafety control measures as outlined by the University's Animal Resource Center.

Bactrim Injection

Eighteen mice were weighed prior to injections, inoculations, and sacrifice. Nine mice each were randomized to either treatment with Bactrim or no treatment. The mice randomized to Bactrim were given daily intraperitoneal injections of the antibiotic (30 mg/kg). After 2 days, all animals were inoculated intranasally with a broth suspension of *S. pneumoniae,* which was susceptible to Bactrim.

Inoculations

Colonies from a 24-hour agar culture of *S. pneumoniae* were suspended in Trypticase soy broth (Becton-Dickinson Microbiology System, Cockeysville, MD) to a turbidity equivalent to a #3 McFarland standard, which corresponds to 1.2×10^9 CFU (colony-forming units)/ml. The animals were sedated with 80 mg/kg ketamine and 8 mg/kg xylazine by intraperitoneal injection. Intranasal inoculation was achieved by placement of 0.02-0.025 ml of the broth containing *S. pneumoniae* ($2.4\text{-}3.0 \times 10^7$ CFU) into the external nares. The broth was drawn into the nasal passages when the animals inhaled. The ATCC strain of *S. pneumoniae* was obtained from the Clinical Microbiology Laboratories of the University of Chicago Hospital. We carefully monitored the breathing rate and skin color of the mice during fluid inhalation to prevent respiratory failure during inoculation. Despite these precautions, 2 mice in the control group died of asphyxia.

Termination Procedure

Five days after bacterial inoculation, the mice were sedated with a respiratory failure dose of Pentobarbital, 120 mg/kg, given by intraperitoneal injection. After sedation, the external nares, oral cavity, and head were disinfected with a very moist alcohol swab and allowed to dry. Sterile normal saline (0.1-0.2 ml) was then used for lavage of the nasal cavities. The lavage liquid was allowed to drip out directly from the nares onto Columbia blood agar and chocolate agar plates. Culture results were graded according to standard microbiological techniques. The nasal lavage sample was streaked for isolation across 3 areas of the plate. *S. pneumoniae* growth on the first streak only was graded as "few colonies", growth on the first and second streaks as "moderate colonies", and growth on the first, second, and third streaks as "many colonies".

After the nasal culture was obtained, the thoracic cavity was quickly opened to provide exposure of the still beating heart. An incision was made in the left ventricle, and a 25-gauge blunt butterfly needle was inserted, with care taken not to damage the intraventricular septum. An incision was then made in the right atrium, and the intravascular system was flushed of blood with lactated Ringer's solution until the liver had blanched and blood no longer drained from the atrium. The mouse was then perfused through the butterfly catheter in the left ventricle with fixative (4% paraformaldehyde and 0.5% glutaraldehyde in a 0.1 M phosphate buffer). The mice were decapitated after the transcardial perfusion.

Histologic Preparation

The heads were soaked in fixative overnight. They were then stripped of eyes, skin, and muscle under low magnification, and the mandible and tongue were discarded. The heads were decalcified overnight in Surgipath Decalcifier II (Rockford, IL). They were trimmed with a fresh razor blade, with excision of the anterior portion of the nose and the brain, leaving a portion containing the nasal sinus approximately 8 mm in length from anterior to posterior. The resulting blocks were embedded in paraffin, sectioned anterior to posterior at 5 mm thickness, and stained with hematoxylin and eosin.

Microscopic Evaluation

Three anatomically similar sections from each mouse were chosen for analysis. The first section, the most anterior, was at the level of the maxillary sinuses; the second, more posterior, was at the end of the maxillary sinuses and the beginning of the complex ethmoid turbinals; and the third, most posterior section contained the brain superiorly. The percent of the sinus cavities occupied by neutrophil clusters and the density of neutrophils infiltrating the mucosa (400× magnification), as represented from a sampling of four mucosal areas, were determined for each of the three sections per mouse with the help of a computer-guided microscope with Neurolucida software (Micobrightfield, Vermont). All tissue sections were examined blindly with respect to the source of the tissue.

Statistics: Data were analyzed by unpaired Student's *t* test and Mann-Whitney *U* test. Numbers are expressed as mean ± standard deviation of the mean. $P<0.05$ was considered to indicate significance.

RESULTS

We evaluated the mice on day 5 after inoculation, a time previously shown to represent the peak of the inflammatory response. The representative results for neutrophil clusters (Figure 1) and for neutrophils per square millimeter of mucosa (Figure 2) are shown. A significantly lower percentage of the sinus cavity area was occupied by neutrophil clusters in the Bactrim-treated group ($1.58\pm1.13\%$, $P<0.05$) than in the control

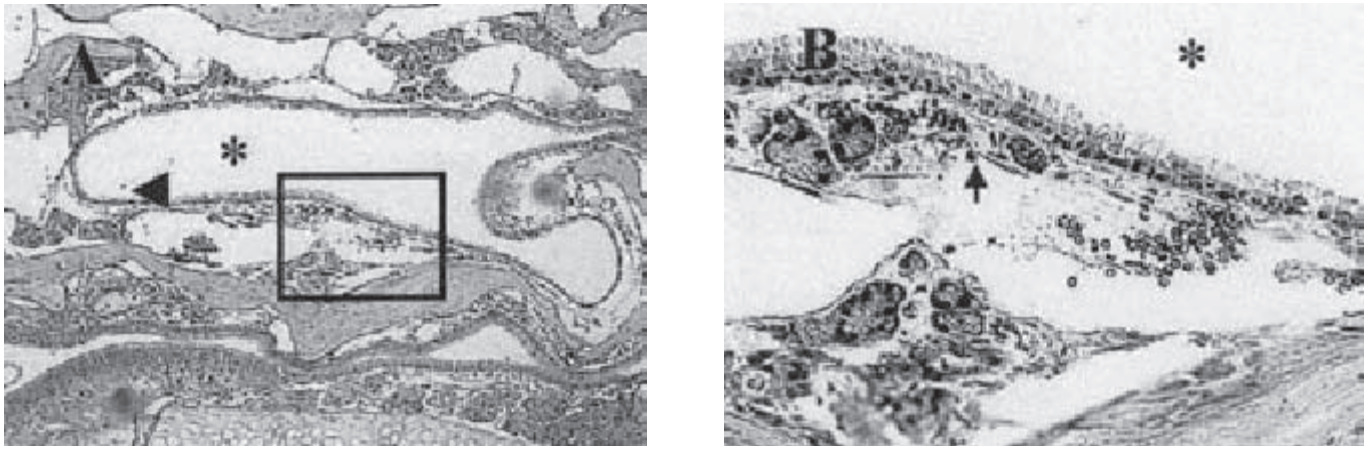

Figure 1. Bactrim-treated mice showed few neutrophil clusters (arrowhead) [Panel A] within the sinus air spaces (asterisk) and few infiltrating neutrophils in the sinus mucosa (arrow) [Panel B]. The epithelium of the sinus mucosa was thin, and cilia were long and regular. H & E stain. Magnification for A=100×, B=400×.

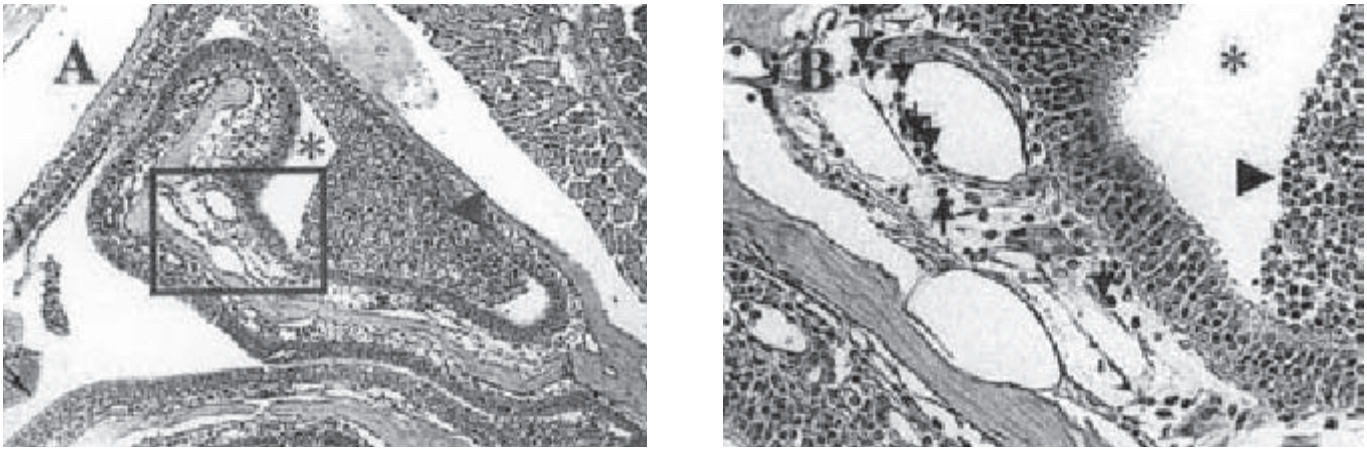

Figure 2. Control mice showed areas of neutrophil clusters (arrowhead) [Panel A] within the sinus air spaces (asterisk) and many infiltrating neutrophils in the sinus mucosa (arrow) [Panel B]. The epithelium of the sinus mucosa was thick, and cilia were irregular. H & E stain. Magnification for A=100×, B=400×.

Table 1. Culture density of *S. pneumoniae* in the Bactrim-treated group and in controls

group/density	few	moderate	many	total
*Bactrim	8	1	0	9
Control	3	3	1	7

* P=0.05

group (4.38±3.41%). Similarly, the number of infiltrating neutrophils in the sinus mucosa was significantly lower in the Bactrim-treated group (58.81±3.41 neutrophils/mm^2, P<0.05) than in the control group (105.85±48.49 neutrophils/mm^2). Culture results showed that lavage fluid from 8 mice had few colonies and 1 had moderate colonies of *S. pneumoniae* in the Bactrim-treated group. Three lavage specimens had few colonies, 3 moderate colonies, and 1 many colonies in the control group (Table 1). This also showed significantly less growth in the Bactrim-treated group than in the control group (P=0.05).

DISCUSSION

We developed a murine model of acute bacterial rhinosinusitis because it was simple to perform, was more physiologic, was rhinologic in origin, and offered a greater potential to study inflammation. Although the anatomy of the mouse sinuses is not identical to that of man, mice possess a similar respiratory epithelium and their sinus cavities are comprised of complex ethmoid turbinals that drain from small openings (Bomer K et al., 1998), as do human sinuses (Wagenmann M, Naclerio RM 1992).

We studied *S. pneumoniae* because it is the most common bacterium cultured from the maxillary sinuses (Evans FO Jr et al., 1975; Malow JB, 1989) of man during acute infection. We chose Bactrim as an antibiotic because it is effective against the strain of *S. pneumoniae* used in our experiments and because it is frequently used for treatment of mice even though it is a bacteriostatic antibiotic with poor penetration into abscess cavities (Williams JW Jr et al., 1995).

The reason for our investigation of mice on day 5 after inoculation was based on prior evidence that the inflammatory response to the infection peaks on that day. The pneumococcal cell

wall-associated antigens are probably responsible for the induction of the initial inflammatory response. Complement activation in response to infection could recruit and activate neutrophils that release neutrophil elastase and cathepsin G, causing desquamation of the adjoining epithelium (Heck LW et al., 1990). The humoral response to *S. pneumoniae* may then play a role in limiting the infection.

We injected Bactrim 2 days before inoculating *S. pneumoniae* in an attempt to increase its effectiveness and to support the role of bacteria in our model of sinusitis. Studying the response when Bactrim is given after the onset of infection, however, would better parallel the situation in man.

CONCLUSION

In our study, Bactrim reduced the degree of *S. pneumoniae* infection in mice. Histologic evidence of reduced inflammation was shown by the decreased area of the sinuses occupied by neutrophil clusters and a decrease in the number of neutrophils infiltrating the sinus mucosa. Decreased infection was evidenced by fewer organisms obtained at culture. These results strengthen our previous observation that *S. pneumoniae* is causing the infection and inflammation in mice. We speculate that our murine model can be used to study the pathophysiology and treatment of acute rhinosinusitis.

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