The Efficacy of Clarithromycin and the Bicyclolide EDP-420 against *Mycobacterium avium* in a Mouse Model of Pulmonary Infection

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Lung disease caused by *Mycobacterium avium* complex (MAC) is increasing in prevalence. MAC disease occurs in patients with chronic preexisting obstructive pulmonary diseases but is also diagnosed in individuals with no history of lung pathology or identifiable immune defect. Histologically, the disease is characterized by either the development of nodular granulomatous lesions in the peribronchial region or cavitary peripheral disease in smokers. Response to long-term treatment is poor. Limited comparative-efficacy data on treatment exist. A model that resembles nodular MAC disease was established in C57 (bg /H11545 bg /H11545) mice infected intranasally. Therapy with clarithromycin, a compound commonly used to treat MAC disease, was evaluated in parallel with treatment using a new bicyclolide, EDP-420, that achieves high levels of intrapulmonary concentrations. Although clarithromycin administered daily resulted in a reduction in the bacterial load in the lung, EDP-420 administered either daily or twice a week was significantly more effective. These results suggest that this animal model can be used to evaluate novel regimens against MAC disease and that compounds with high concentration in the lung might have a significant impact on the outcome of MAC lung disease.

Lung disease caused by organisms of the *Mycobacterium avium* complex (MAC) has been increasingly recognized [1, 2]. MAC is a pathogen commonly isolated from environmental sources, such as water and soil [3, 4]. The bacterium can be isolated from potting soil as well as from urban water pipes, which are potential sources of exposure [5, 6]. Disseminated MAC disease has occurred among immune-impaired individuals, such as those suffering from advanced AIDS [7, 8]. MAC lung disease was originally described in patients with prior lung damage but more recently has been observed in the lung tissue of individuals with no defined immune defect or history of lung abnormality [9, 10]. In addition, MAC infection of the lung tissue of patients with cystic fibrosis has become increasingly common [11]. The disease is characterized by the slow development of inflammation and later by granulomatous lesions in the peribronchial region [12]. The clinical course is insidious, with women being more frequently affected than men [13].

Recent studies suggest that the ability to form biofilm in the airways may be related to the establishment of infection [14]. MAC strains deficient in biofilm formation are less capable of causing pulmonary infection in a mouse model [14]. Infection caused by other microorganisms, such as *Pseudomonas aeruginosa*, are also linked to the ability to develop biofilm [15].

Although the infection initially responds to combination therapy with a macrolide and ethambutol (and often a third or fourth drug), recurrence and eventual development of resistance to antibiotics is not uncommon. It is assumed that infections treated for <10 weeks have an increased chance to recur with the same organism...
The clinical presentation of the disease can be either cavitary or nodular, with the cavitary form often associated with smoking and the nodular being associated with chest deformities in women [17]. Several recent observations indicate that prognosis is more reserved in patients with cavitary disease than in patients with nodular disease [13, 18].

The ability to evaluate potential therapy for treatment of MAC infection in patients with lung disease has not been fully explored in an animal model, because of the absence of a model that reproduces the pathology seen in humans. An animal model that resembles the infection in humans is needed for the development of improved therapeutic regimens. We have established a model in which the infection follows crossing of the bronchial mucosa by the bacterium [14], resulting in an organized granulomatous response in the peribronchial area [14]. In the present report, we describe the evolution of the infection in the model and evaluate the efficacy of clarithromycin, a compound frequently used to treat pulmonary infection caused by MAC. In addition, we compare the efficacy of clarithromycin to a new compound, in the bicyclolide class, that achieves very high concentrations in lung tissue [19].

**MATERIAL AND METHODS**

**Challenge Organisms.** *Mycobacterium avium* strain 101, an isolate from the blood of a patient with AIDS, was cultured, for 10 days at 37°C, on Middlebrook 7H11 agar plates containing 10% oleic acid, albumin dextrose, and catalase (OADC). Before the infection, organisms were suspended in Hanks’ balanced salt solution and were adjusted to a concentration of $8 \times 10^8$ cfu/mL. The suspension was plated onto Middlebrook 7H11 agar plates containing 10% OADC, to determine the number of bacteria in the inoculum.

**Mice and Infection.** Female C57 BL/6 (bg/bg) beige mice weighing ~20 g and obtained from Jackson Laboratories in Bar Harbor, Maine, were used after a 1-week quarantine period. The mice were briefly anesthetized with isofluorane, and a micropipette was used to introduce $2 \mu$L of $8 \times 10^8$ cfu/mL suspension introduced into each animal’s nostrils. The mice were observed for several weeks and were killed at biweekly intervals, at 9–19 weeks after challenge. Blood samples were collected and analyzed by radiometric BACTEC system, as reported elsewhere [20]. Spleens and lungs were aseptically dissected, weighed, and then homogenized in 3 mL of Middlebrook 7H9 broth containing 20% glycerol. Homogenates were serially diluted and then were plated onto Middlebrook 7H11 plates containing 10% OADC, to determine the number of bacteria in the inoculum.

**Antibiotics and Therapy Protocol.** Clarithromycin was obtained commercially, and EDP-420 was a gift from Enanta Pharmaceuticals, Inc. The compounds were prepared as described elsewhere [21], by suspending the agents in 2.5% gum Arabic with 0.05% Tween 80. Lung and spleen tissue from the mice was harvested 3 weeks after infection, to quantify pretreatment bacterial load in their lungs and spleens. Then the mice were treated with either EDP-420 (100 mg/kg) or clarithromycin (100 mg/kg), either every day or twice a week. These doses were chosen on the basis of previous experience determining the effective dose [22, 23]. Control mice received no treatment except the vehicle (i.e., 2.5% gum Arabic with 0.05% Tween 80). After 4 weeks of therapy, all control mice were killed, and their spleens and lungs were aseptically dissected, weighed, and homogenized in 3 mL of Middlebrook 7H9 broth containing 20% glycerol. Homogenates were serially diluted and plated onto 7H11 agar plates containing 10% OADC. Bacteria were allowed to grow for ~10 days before being counted.

**Biofilm Treatment.** Biofilm appears to play a significant role in the establishment of MAC infection in the lung, as has been demonstrated in an animal model [14]. To establish whether the compounds were able to kill MAC once it is in biofilm, we seeded MAC 101 onto polyvinylchloride plates, as described elsewhere [24, 25], and, at day 0 (seeding time), day 4, and day 7, either clarithromycin or EDP-420 was added at a subinhibitory concentration of either 2 μg/mL or 1 μg/mL, re-
respectively. Biofilm mass was measured at day 14. In subsequent experiments, both compounds were added, at a concentration of 2 g/mL (i.e., their MICs) to established biofilms (day 7 after seeding). The biofilm mass was measured at day 14, as described elsewhere [24, 25].

Statistics. Experiments were repeated at least twice, and the results were analyzed by comparing the experimental groups with the control groups. Student’s t-test was used to verify the significance of the results; P < .05 was considered to be statistically significant.

RESULTS

Development of Lung Disease. At 3 weeks after challenge, the lung bacterial load was ~3 × 10⁵ bacteria/g of tissue. During the ensuing several weeks, the count increased to ~1.4 × 10⁷ bacteria/g of lung and ~3 × 10⁷ bacteria/gram of lung (figure 1A). By 19 weeks, gross lung weights had increased by an average of 50%. Histopathological changes characterized by saccular bronchiectasis, granulomas, intense monocytic-lymphocytic infiltration, and hemorrhage were observed at different time points after 7 weeks of infection (figure 2A–2D). The lung parenchyma of >90% of beige mice can be predictably infected via a single nasal inoculation into both nostrils [14]. Splenic enlargement was observed late in the infection, but bacteremia was not detected by week 19. Figure 1A and figure 1B show the number of bacteria in lung and spleen tissue, respectively. The disease was progressive, with no growth plateau occurring up to week 19. Marked inflammatory changes, as well as peribronchial inflammatory infiltrate, were observed.

<table>
<thead>
<tr>
<th>Day when antibiotic was added</th>
<th>EDP-420</th>
<th>Clarithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% MIC</td>
<td>MIC 2 µg/L</td>
</tr>
<tr>
<td>0</td>
<td>22 ± 6ᵃ</td>
<td>1 ± 0.4ᵃ</td>
</tr>
<tr>
<td>4</td>
<td>38 ± 3ᵃ</td>
<td>21 ± 8ᵃ</td>
</tr>
<tr>
<td>7</td>
<td>98 ± 2</td>
<td>64 ± 7ᵇ</td>
</tr>
</tbody>
</table>

NOTE. Data are % of biofilm formation; when no antibiotic was added, biofilm formation was 100%. ᵃ P < .05, compared with results when no antibiotic was added (i.e., 100% biofilm formation). ᵇ P < .05, compared with results when clarithromycin at MIC was added at day 7 after seeding.
Table 2. Effect of clarithromycin and EDP-420 regimen on lung and spleen from *Mycobacterium avium* complex–infected mice.

<table>
<thead>
<tr>
<th>Treatment status</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (3 weeks</td>
<td>5.9 ± 1.2 × 10^6</td>
<td>1.2 ± 0.5 × 10^3</td>
</tr>
<tr>
<td>after challenge)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment (controls)</td>
<td>8.0 ± 1.6 × 10^6</td>
<td>4.4 ± 1.4 × 10^4</td>
</tr>
<tr>
<td>Clarithromycin (100 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>2.0 ± 0.5 × 10^{10b}</td>
<td>1.2 ± 0.4 × 10^{10b}</td>
</tr>
<tr>
<td>Biweekly</td>
<td>4.3 ± 0.7 × 10^{10b}</td>
<td>1.6 ± 0.3 × 10^{10b}</td>
</tr>
<tr>
<td>EDP-420 (100 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>3.1 ± 0.5 × 10^{10a,b,c}</td>
<td>6.7 ± 0.4 × 10^{10a,b,c}</td>
</tr>
<tr>
<td>Biweekly</td>
<td>7.4 ± 1.0 × 10^{10a,b,c}</td>
<td>1.5 ± 0.7 × 10^{10a,b,c}</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD colony-forming units per organ; results were similar (−log) when colony-forming units per gram of tissue was the concentration used.

a  *P* < .05, compared with baseline values.

b  *P* < .05, compared with untreated controls (7 weeks after challenge).

c  *P* < .05, compared with clarithromycin administered on the same schedule.

**Response to Therapy.** To evaluate the action of antibiotics in this model, we treated mice with clarithromycin, a drug commonly used to treat MAC infection (including lung infection), and with EDP-420, an experimental drug (currently in clinical trial for treatment of community-acquired pneumonia) that achieves very high levels in the lung [19]. Daily treatment for 4 weeks, with either of these 2 drugs, resulted in a significant decrease in the number of bacteria in the lungs and in the spleen (table 1). Because many patients with MAC infection require long-term treatment, we also evaluated the response of the infection to the antibiotics when the latter were administered twice a week. As seen in table 2, although treatment with EDP-420 was associated with a significant reduction in the bacterial load, treatment with clarithromycin did not have a significant effect on the infection.

**Effect of EDP-420 on MAC biofilm.** To investigate whether EDP-420’s effect on MAC biofilm was different than that of clarithromycin [24], we established MAC biofilm on polystyrene–infected plates and, at different time points after bacterial seeding, exposed them to subinhibitory concentrations of EDP-420. As shown in table 1, subinhibitory concentrations of EDP-420, like subinhibitory concentrations of clarithromycin, are capable of interfering with biofilm formation when they are added at day 0 and day 4 after seeding but not when added at day 7 after seeding. When added to MAC biofilm at day 7, the MIC of EDP-420 had a significant effect on bacterial viability, whereas the MIC of clarithromycin did not (table 1).

**DISCUSSION**

Lung infection caused by MAC is an increasing problem in populations of risk, as well as in individuals without any known predisposing factors [1, 2, 4]. Initially, treatment is usually effective, but recurrence is a common feature in this population of patients [1, 26]. Recent work has suggested that biofilm formation plays a role during the MAC colonization in inert material [14], and it has been reported that the use in vivo of mutants that are impaired in biofilm formation results in a significant decrease in the level of infection in mice [14]. The chronic nature of the infection represents a challenge for therapy [1, 26]. Frequently, treatment with initially effective antibiotics results in the development of resistance to the effective drugs [1, 26]. Combination therapy with a macrolide, usually clarithromycin plus either ethambutol or rifabutin, is associated with increased efficacy and decreased emergence of resistance [27]. Despite the current therapeutic shortcomings, no experimental system has been used to evaluate novel regimens.

A model has been developed in which the pathology resembles the infection—namely, noncavitary bronchocentric disease—in the great majority of nonsmoking individuals. In this model, experimental therapy can be evaluated. Other approaches have been used to establish *M. avium* infection in the lung, with the bacteria being delivered via aerosol [28]. In the present study, we have confirmed the efficacy of clarithromycin in the treatment of MAC lung infection and have shown that, as a treatment of the infection, EDP-420, a bicyclolide, is significantly more active than clarithromycin. In addition, when the drugs were administered twice weekly, treatment with EDP-420 resulted in significant activity, whereas clarithromycin resulted in no activity. Although not commonly used, intermittent therapy for MAC lung disease has several potential advantages, from its lower toxicity to its decreased cost. A couple of clinical trials have reported a good response, except when the patients had cavitary disease or significant bronchiectasis [1, 27].

The challenge organism used in these studies is inhibited by clarithromycin in vitro at 2 μg/mL, whereas the MIC of EDP-420 in vitro is 4 μg/mL. EDP-420, however, had a greater bactericidal effect than did clarithromycin. Possible explanations of why EDP-420 results in increased MAC killing are that it (1) achieves high concentrations lung tissue and (2) is able to kill bacteria in biofilms. EDP-420 has been tested in healthy subjects and, in the epithelial lining fluid, has been found to have a half-life of 14.8 h, with a C_{max} of 16.8 μg/mL. In alveolar macrophages, the C_{max} concentration has been found to be 175.9 μg/mL. In plasma, the mean half-life was 15.6–20.1 h [29, 30]. In vitro, when a subinhibitory concentration was delivered to established biofilms, both EDP-420 and clarithromycin had limited activity, which was restricted to the period when the biofilm was partially established [24]. At MIC, however, EDP-420, in contrast to clarithromycin, had anti-MAC activity. Why the activity was different at MIC but not at subinhibitory concentration remains unknown; however, it might be important for the treatment of lung infection. It has been suggested that, in mice, biofilm formation plays an important role in the establishment...
of MAC lung disease [14]. Future studies must establish whether there is in the biofilm a MAC subpopulation (planktonic vs. sessile) that is susceptible to inhibitory concentrations of EDP-420. P. aeruginosa in biofilm has been shown to display tolerance to antibiotics, but killing by colistin (a cyclic cationic decapetide) has been to be more efficient in the inner part of the biofilm than against planktonic cells [31].

In summary, we have described a model of MAC lung infection, a model that can be used to evaluate response to therapy—and to evaluate it in a way that is potentially more relevant for MAC lung disease. The results of the present study suggest that drugs that achieve especially high concentrations in the lung and/or that are active against the biofilm form of MAC might play a role in the treatment of the infection in humans.

Acknowledgments

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References