In vitro activity of cethromycin, a novel antibacterial ketolide, against Chlamydia pneumoniae

Naoyuki Miyashita*, Hiroshi Fukano, Koichiro Yoshida, Yoshihito Niki and Toshiharu Matsushima

Division of Respiratory Diseases, Department of Medicine, Kawasaki Medical School, 577 Matsushima, Kurashiki City, Okayama 701-0192, Japan

Received 14 February 2003; returned 15 April 2003; revised 13 June 2003; accepted 17 June 2003

Objectives: To investigate the in vitro activity of cethromycin, a new ketolide, against Chlamydia pneumoniae.

Methods: The in vitro activity of cethromycin against 20 isolates of C. pneumoniae was compared with the activities of telithromycin, erythromycin A, azithromycin and clarithromycin against those isolates.

Results: The MIC at which 90% of the isolates were inhibited and the minimal chlamydiacidal concentration at which 90% of the isolates were killed by cethromycin were both 0.016 mg/L (range 0.016–0.031 mg/L). Cethromycin was the most active antibiotic tested in this study.

Conclusions: Our results appear to indicate that cethromycin is an effective antibiotic that should play some role in the treatment of respiratory tract infections caused by C. pneumoniae.

Keywords: macrolides, telithromycin, community-acquired pneumonia

Introduction

The ketolide class of antibacterial agents includes 14-membered ring macrolides that differ from erythromycin A in that they have a 3-keto group instead of the L-cladinose moiety in the lactone ring. Telithromycin was the first ketolide to be developed for clinical use. Another ketolide, cethromycin (formerly ABT-773), has a cyclic carbamate group at the 11, 12-position in addition to the 3-keto group. This antibiotic agent exhibits good antibacterial activity against a broad range of respiratory pathogens, including multiresistant Streptococcus pneumoniae and staphylococci, Haemophilus influenzae, Moraxella catarrhalis, Legionella spp. and Mycoplasma pneumoniae. Chlamydia pneumoniae is recognized as an important pathogen of respiratory tract infections worldwide, is a common cause of pneumonia, bronchitis, pharyngitis and sinusitis, and is responsible for almost 10% of cases of community-acquired pneumonia (CAP). The in vitro activity of macrolides and telithromycin against C. pneumoniae is variable, with clarithromycin showing the lowest MICs followed by telithromycin, azithromycin and erythromycin A. However, the available data on the activity of cethromycin against C. pneumoniae are limited. Therefore, we compared the in vitro activities of cethromycin and other macrolides and telithromycin against five standard strains and 15 wild-type Japanese isolates of C. pneumoniae.

Materials and methods

Antimicrobial agents

The antimicrobial agents tested were erythromycin A (Shionogi Co., Osaka, Japan), clarithromycin (Taisho Pharmaceutical Co., Osaka, Japan), azithromycin (Pfizer Pharmaceutical Co., Tokyo, Japan), telithromycin (Aventis Pharma Co., Tokyo, Japan) and cethromycin (Dainabot Laboratories, Osaka, Japan). Solutions of the agents were prepared following the manufacturers’ instructions.

Isolates

Twenty C. pneumoniae isolates were used in this study. TW-183, AR-39 and AR-388 were obtained from the Washington Research Foundation, Seattle, WA, USA. JOL-207 and Kajaani-6 were acquired from P. Saikku, National Public Health Institute, Oulu, Finland. Fifteen wild-type isolates (designated KKn-1 to KKn-15) were also tested, which were isolated from nasopharyngeal swab specimens collected from patients with acute respiratory tract infections at Kawasaki Medical School Hospital, Japan. The organisms from these clinical samples were positively stained with C. pneumoniae-specific monoclonal antibody. These clinical isolates were morphologically different from TWAR (TW-183, AR-39 and AR-388) strains from the United States (examples are given in Figure 1).

KKn-15 elementary bodies (EBs) have a narrow periplasmic space and...
are round in shape, whereas TW-183 EBs are enclosed by a wavy outer membrane and are ‘pear-shaped’ in profile.

**Measurement of MICs and minimal chlamydiacidal concentrations (MCCs)**

One millilitre of culture medium [Eagle’s minimal essential medium (Nissui Pharmaceuticals Co., Tokyo, Japan) and 10% heat-inactivated fetal calf serum (GIBCO BRL Life Technologies Inc., Grand Island, NY, USA)] containing $10^5$ HEp-2 cells per mL were dispensed into each well of plastic 24-well culture plates, which were then incubated in 5% CO$_2$ at 35°C for 48 h. After confirming growth of a confluent monolayer, the culture fluid was removed from the wells by aspiration. Next, $10^4$ inclusion-forming units per mL of each chlamydial strain were inoculated into each well. Then the plates were centrifuged at 900 g for 60 min, and 1 mL of each preparation of the culture medium containing 1 mg/L cycloserine (Nakarai Tesque Inc., Tokyo, Japan) and one concentration (final concentrations; range from 0.008 to 2 mg/L) of the test antibiotic were dispensed into each well. After incubation in 5% CO$_2$ at 35°C for 72 h, the cultures were fixed and stained for inclusions with the fluorescein isothiocyanate-conjugated monoclonal antibody specific for the chlamydial genus-specific antigen (Chlamydia FA Seiken; Denka Seiken, Tokyo, Japan). The MIC was defined as the lowest concentration at which no inclusions were found. The MCCs were determined by aspirating the antibiotic-containing medium, washing the wells twice with phosphate-buffered saline, and adding antibiotic-free medium. The infected cells were frozen at −70°C, thawed, passed onto new cells, incubated for 72 h, and then fixed and stained as described above. The MCC was the lowest antibiotic concentration resulting in no inclusions after passage. All tests were run in triplicate. Antichlamydial activity was determined when the same results were observed in at least two out of three experiments.

**Results**

The MIC and MCC ranges of cethromycin and the other antimicrobial agents for *C. pneumoniae* used in this study are shown in Table 1. The MICs and MCCs of cethromycin for the 20 *C. pneumoniae* isolates both ranged between 0.016 and 0.031 mg/L. The MICs of cethromycin, telithromycin, clarithromycin, azithromycin and erythromycin A at which 90% of the isolates were inhibited (MIC$_{90}$) were 0.016, 0.063, 0.063, 0.25 and 0.25 mg/L, respectively. The MCC$_{90}$ of cethromycin was also 0.016 mg/L. Cethromycin was the most active antibiotic tested in this study.

**Discussion**

*C. pneumoniae* is a well-known respiratory pathogen that causes upper and lower respiratory tract infections and pneumonia. Macrolides and ketolides have been demonstrated to be active *in vitro* against *C. pneumoniae*. We have previously reported on the experimental effectiveness of macrolides against acute chlamydial respiratory tract infections. The therapeutic effect of a 7 day course of clarithromycin at doses of 5 and 10 mg/kg body weight administered orally twice daily and of azithromycin at a dose of 10 mg/kg body weight administered orally once daily to mice with experimental *Chlamydia psittaci* pneumonia was excellent, with a 100% survival rate at 14 or 21 days after infection. This finding was the same as that for treatment with minocycline administered at 10 mg/kg twice daily. Recently, Hammerschlag et al. reported a study assessing the efficacy of cethromycin for treatment of *C. pneumoniae* pneumonia. They found a 100% efficacy in eradication of the organism from the nasopharynx of patients with CAP. Telithromycin (given at a dosage of 800 mg once daily for 7–10 days) also showed good clinical efficacy against CAP due to *C. pneumoniae*. However, the diagnosis of *C. pneumoniae* infection in these telithromycin studies was based entirely on serology, not culture. Clinical studies on erythromycin A, clarithromycin and azithromycin in which cultures were carried out demonstrated that these macrolides are effective drugs for the treatment of respiratory infection associated with *C. pneumoniae*.

The available data on the activity of cethromycin against *C. pneumoniae* are limited. Strigel et al. found that both the MIC$_{90}$ and MCC$_{90}$ values of cethromycin against 20 isolates of *C. pneumoniae* including two reference strains, TW-183 and AR-39, were 0.015 mg/L. The MIC$_{90}$ and MCC$_{90}$ against 20 isolates of *C. pneumoniae* found in our study are also consistent with their report. Based on the above

**Table 1. In vitro activities of cethromycin and other antimicrobial agents against 20 isolates of Chlamydoaphila pneumoniae**

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (mg/L)</th>
<th>MCC (mg/L)</th>
<th>Agent</th>
<th>MIC (mg/L)</th>
<th>MCC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
<td>range</td>
<td>MCC$_{90}$</td>
</tr>
<tr>
<td>Cethromycin</td>
<td>0.016–0.031</td>
<td>0.016</td>
<td>0.016</td>
<td>0.016–0.031</td>
<td>0.016</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.031–0.25</td>
<td>0.063</td>
<td>0.063</td>
<td>0.031–0.25</td>
<td>0.063</td>
</tr>
<tr>
<td>Erythromycin A</td>
<td>0.063–0.25</td>
<td>0.125</td>
<td>0.25</td>
<td>0.063–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.125–0.5</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125–0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.016–0.063</td>
<td>0.031</td>
<td>0.063</td>
<td>0.016–0.063</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Downloaded from https://academic.oup.com/jac/article-abstract/52/3/497/776045 by guest on 22 December 2018
findings and previous reports of the potent and broad antibacterial activity of cethromycin, we can conclude that cethromycin, like telithromycin, could be a useful oral agent for the acute treatment of respiratory tract infections. Prospective studies of cethromycin for the treatment of CAP should be able to determine the role of this drug in the treatment of such infections.

Acknowledgements

This work was supported by a Project Research Grant from Kawasaki Medical School (13-401, 14-402, 15-405A).

References