Activity of clarithromycin alone and in combination in a murine model of Mycobacterium kansasii infection

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Activities of clarithromycin alone and in combination with rifampicin, gatifloxacin or linezolid were evaluated against Mycobacterium kansasii in a murine infection model. Clarithromycin was the most active single agent. Rifampicin and gatifloxacin had similar activities, but were less active than clarithromycin. Clarithromycin in combination with rifampicin was the most active combination therapy.

Keywords: clarithromycin, chemotherapy, combination therapy, M. kansasii, murine infection model, mycobacteria

Introduction

Current therapy for Mycobacterium kansasii infection includes rifampicin, and ethambutol with or without isoniazid.1–4 Rifampicin is the cornerstone in these regimens.2–5,6 Clarithromycin and azithromycin have demonstrated promising activity against non-tuberculous mycobacteria (including M. kansasii) in a murine infection model.7 Clarithromycin has proved to be active in vitro and in vivo against Mycobacterium avium.8–10 Gatifloxacin is an 8-methoxy quinolone with good in vitro activity against Mycobacterium tuberculosis,11 and linezolid is an oxazolidinone with modest in vitro activity against M. tuberculosis.12 The purpose of the current study was to evaluate the activities of clarithromycin alone and in combination with gatifloxacin, linezolid or rifampicin in a murine model of M. kansasii infection.

Materials and methods

Five- to 6-week-old female beige mice, strain C57BL/6J-Lystbg+/- (Jackson Laboratory, Bar Harbor, ME, USA), were anaesthetized with ketamine/xylazine mixture IM (ketamine 45 mg/kg (Fort Dodge Animal Health, Fort Dodge, IA, USA) and xylazine 7.5 mg/kg (Bayer Corporation, Shawnee Mission, KS, USA)). Mice were infected with 20×106 viable M. kansasii 795 (a clinical isolate from the VAMC, Syracuse, NY, USA). The inoculum size was verified by plating serial dilutions of the bacterial suspension in triplicate on 7H10 agar plates (BBL Microbiology Systems, Cockeysville, MD, USA) with 10% oleic acid–albumin–dextrose–catalase (OADC) enrichment. Colonies of M. kansasii were counted after 4 weeks of incubation at 37°C in ambient air.

Each treatment group contained six mice. Treatment began 1 week post-infection, with drugs administered orally by gavage 5 days per week for 4 weeks. The following treatment groups were included: untreated early and late controls, gatifloxacin (100 mg/kg), clarithromycin (200 mg/kg), linezolid (100 mg/kg), gatifloxacin + clarithromycin, clarithromycin + linezolid, gatifloxacin + linezolid, rifampicin (20 mg/kg) and rifampicin + clarithromycin. Bristol-Myers Squibb (Princeton, NJ, USA) provided gatifloxacin, Abbott Laboratories (Abbott Park, IL, USA) clarithromycin, Pharmacia (Kalamazoo, MI, USA) linezolid, and Sigma Chemical Company (St Louis, MO, USA) rifampicin. Broth dilution MICs for M. kansasii 795 were as follows: 0.25, 2, 4 and 0.25 mg/L of gatifloxacin, clarithromycin, linezolid and rifampicin, respectively. Gatifloxacin and clarithromycin were dissolved in ethanol and distilled deionized water (2:8, v/v). Linezolid and rifampicin were dissolved in dimethyl sulfoxide and distilled deionized water (2:8, v/v). Drugs were delivered in 0.2 mL of vehicle. Mice receiving combination therapy were dosed in the mornings and afternoons.

The early control group was euthanized by CO2 inhalation 1 week post-infection. The late control group was euthanized at the end of the 4 week treatment period. Right lungs were harvested, ground in a tissue homogenizer (IdeaWorks Laboratory Devices, Syracuse, NY, USA), and plated onto 7H10 agar plates (BBL Microbiology Systems, Cockeysville, MD, USA) with 10% oleic acid–albumin–dextrose–catalase (OADC) enrichment. Colonies of M. kansasii were counted after 4 weeks of incubation at 37°C in ambient air.

This study was conducted in accordance with the ethical standards for animal experimentation of the Department of Veterans Affairs and our Institutional Animal Care and Use Committee.

A Kruskal–Wallis analysis of variance produced an H value of 44.78 with 9 df, P < 0.001. This implies that there were statistically significant differences between the groups. To determine which group differed from another, the Mann–Whitney non-parametric test was used.

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Clarithromycin in *Mycobacterium kansasii* infection

<table>
<thead>
<tr>
<th>Group (no. of mice)</th>
<th>Log$_{10}$ cfu [median (90% confidence interval)]</th>
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<tbody>
<tr>
<td>Early controls (6)</td>
<td>7.46 (7.13–7.75)</td>
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<tr>
<td>Late controls (6)</td>
<td>8.78 (8.36–8.97)</td>
</tr>
<tr>
<td>GAT 100 mg/kg (5)$^a$</td>
<td>6.51 (6.07–6.85)</td>
</tr>
<tr>
<td>CLR 200 mg/kg (5)$^a$</td>
<td>5.53 (4.69–5.87)</td>
</tr>
<tr>
<td>GAT + CLR (6)</td>
<td>5.89 (5.39–6.03)</td>
</tr>
<tr>
<td>RIF 20 mg/kg (6)</td>
<td>6.33 (5.79–6.89)</td>
</tr>
<tr>
<td>RIF + CLR (5)$^b$</td>
<td>5.04 (4.87–5.08)</td>
</tr>
<tr>
<td>LZD 100 mg/kg (6)</td>
<td>7.04 (6.91–7.12)</td>
</tr>
<tr>
<td>CLR + LZD (6)</td>
<td>5.57 (4.16–5.87)</td>
</tr>
<tr>
<td>GAT + LZD (6)</td>
<td>6.20 (4.33–6.51)</td>
</tr>
</tbody>
</table>

CLR, clarithromycin; GAT, gatifloxacin; LZD, linezolid; RIF, rifampicin.

$^a$The mice missing from these groups died owing to a technical error.

Results and discussion

The late control group was statistically significantly different from the early control group (log cfu counts: 7.46 versus 8.78, respectively; $P = 0.0081$). All drug and drug combinations were significantly different from both the early and late control groups ($P < 0.05$).

In the monotherapy groups, clarithromycin was significantly more active than gatifloxacin (log cfu counts: 5.53 versus 6.51, respectively; $P = 0.022$) and linezolid (log cfu counts: 5.53 versus 7.04, respectively; $P = 0.008$) but it was not statistically different from rifampicin (log cfu counts: 5.53 versus 6.33, respectively; $P = 0.08$). None of the clarithromycin-containing combinations were statistically better than clarithromycin alone (clarithromycin versus clarithromycin + gatifloxacin, log cfu counts: 5.53 versus 5.89 respectively, $P = 0.21$; clarithromycin versus rifampicin + clarithromycin, log cfu counts: 5.53 versus 5.04, respectively, $P = 0.14$; clarithromycin versus clarithromycin + linezolid, log cfu counts: 5.53 versus 5.57, respectively, $P = 1.00$; clarithromycin versus gatifloxacin + linezolid, log cfu counts: 5.53 versus 6.20, respectively, $P = 0.21$). Clarithromycin was the most active agent when used alone or in combination with the other drugs; it reduced organ viable cell counts by approximately 2 logs. Rifampicin demonstrated activity against *M. kansasii* reducing the count by approximately 1 log. Gatifloxacin was as active as rifampicin (log cfu counts: 6.51 versus 6.33, respectively; $P = 0.65$). Linezolid treatment decreased viable cell counts by only one half of 1 log.

All drug combinations performed as well or better than either component drug alone; therefore, there was no evidence of any in vivo antagonistic drug interactions. The best combination was rifampicin + clarithromycin (log cfu count: 5.04). This combination worked better than rifampicin (log cfu counts: 6.33), gatifloxacin (log cfu counts: 6.51) or linezolid (log cfu counts: 7.04) alone, and was also more active than the gatifloxacin + clarithromycin combination (log cfu counts: 5.89).

These results confirmed the favourable activity of clarithromycin and rifampicin against *M. kansasii* in mice. It would be of interest to evaluate further the activity of fluoroquinolones such as gatifloxacin against non-tuberculous mycobacteria including *M. kansasii*. Based on the activities of each of these agents alone, the combination of rifamycin derivatives and fluoroquinolones merits more attention. Additional murine model studies, as well as clinical studies, are necessary to define the most effective and safest way to use clarithromycin in combination to treat infections caused by *M. kansasii* and other non-tuberculous mycobacteria.

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References