Treatment alternatives for Mycobacterium kansasii

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Mycobacterium kansasii was administered intravenously to congenitally athymic (nude) mice. Beginning 1 week later, rifapentine, azithromycin, ethambutol or combined therapy was initiated orally. All three drugs were highly active individually. Although there was no evidence of antagonism, combined therapy was not more effective than either component used alone.

Introduction

Among non-tuberculous mycobacteria recovered from patients with HIV infection, Mycobacterium avium--intracellulare (MAC) accounts for well over 90\%.\textsuperscript{1} Mycobacterium kansasii in some series is second to MAC as a cause of serious non-tuberculous mycobacterial infection in patients with AIDS.\textsuperscript{2,3} Optimal treatment of \textit{M. kansasii} in non-HIV-infected patients includes isoniazid, rifampicin and ethambutol. Patients with AIDS and \textit{M. kansasii} infection are at a disadvantage with this traditional regimen, because rifampicin greatly accelerates hepatic metabolism of HIV protease inhibitors, rendering them ineffective. Accordingly, Wallace \textit{et al.}\textsuperscript{4} and others\textsuperscript{5} have recommended clarithromycin as a secondary agent for the treatment of rifampicin-resistant \textit{M. kansasii}.

In the present study we evaluated two other agents, azithromycin and rifapentine, and compared these with ethambutol, a standard treatment drug. Azithromycin was chosen because it is active \textit{in vitro} against \textit{M. kansasii}, is not a strong cytochrome enzyme inducer and is active \textit{in vivo} in mice.\textsuperscript{5-8} Rifapentine was chosen because it is a less intense hepatic microsomal enzyme inducer than rifampicin, is cleared more slowly than rifampicin and is active against \textit{M. kansasii}. We performed dose ranging for survival studies, explored potential combinations using survival studies and finally conducted a study of tissue burden using one regimen.

Materials and methods

\textit{M. kansasii}

A clinical isolate (Hill) was obtained from a patient with HIV and \textit{M. kansasii} disease. Before infection, colonies were inoculated on to Lowenstein agar for 14 days. The organisms were scraped from the plate, washed three times in sterile isotonic saline, counted in a haemocytometer and adjusted to \(10^7\) organisms per 0.1 mL mouse dose. All inocula were confirmed by serial colony count dilution, and viable counts are reported.

\textit{In vitro testing}

The MIC after a 2 week incubation in Middlebrook 7H9 broth was <0.5 mg/L for rifapentine and 2 mg/L for azithromycin and clarithromycin.\textsuperscript{6,9}

\textit{Animal model}

BALB/c athymic mice were obtained from our breeding colony, which is certified as following the National Institutes of Health Guidelines for Laboratory Animal Medicine. Mice were infected intravenously at approximately 6 weeks of age. In preliminary screening studies, athymic mice lost weight and eventually succumbed between 2 weeks and 2 months after infection. The speed of attrition was dependent on the inoculum dose.

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Treatment regimens

One week after infection, treatment was begun with various drug regimens, with the doses based on prior mouse studies with MAC. As the infecting inoculum was generally in the region of $10^7$ cfu, and because control mice succumbed in a similar time frame, some of the results represent pooled studies. Treatment was continued for 21 days, and mice were observed until day 35 for survival studies. Treatment was given as 0.2 mL gavage, and included rifapentine at 0.15, 0.3 or 0.6 mg/kg/day, ethambutol at 10, 25 or 100 mg/kg/day and azithromycin at 10, 15, 30, 50 or 150 mg/kg/day. Mice were killed on day 35. The spleen and liver were removed under aseptic conditions, homogenized in 2 mL of water and serial 10-fold dilutions were made for colony counts. Cultures were incubated for 2–3 weeks, until colonies were readily visible.

Based on the results of monotherapy survival studies, we tested combinations of suboptimal doses of individual drugs. Because survival studies might be less sensitive than tissue burden to effects of combination therapy, we studied tissue burden. Our intravenous model produces a widely disseminated infection targeting the liver and spleen, and these organs were selected.

Statistics

For survival studies, groups were compared by the Wilcoxon test of life tables. For tissue burden studies non-parametric comparison was carried out using Dunnett’s two-tailed $t$-test. $P < 0.05$ determined significance.

Results

Survival studies were first carried out with individual drugs. Rifapentine was highly active, with a sharp break between effective (0.6 mg/kg, $P < 0.001$ compared with control) and ineffective doses (0.3 mg/kg, $P = 0.11$ compared with control) (Figure 1a). Azithromycin also significantly prolonged survival, down to 15 mg/kg/dose, with $P = 0.02$ compared with control (Figure 1b). Ethambutol significantly prolonged survival down to 10 mg/kg, the lowest dose tested, with $P < 0.001$ compared with controls (Figure 1c).

Doses for combination studies were chosen to reflect the lowest doses showing antimycobacterial activity in the single drug studies. Rifapentine alone was protective (Figure 2a), but was not superior to azithromycin ($P = 0.9$). As shown in Figure 2b, azithromycin and ethambutol did not show any increase in protection over individual drugs ($P = 0.9$ compared with ethambutol alone). A final study of combination therapy was performed with tissue counts of liver and spleen. As shown in Figure 3, rifapentine significantly reduced spleen ($P = 0.002$) and liver ($P = 0.026$) tissue burden. Azithromycin was ineffective used alone in spleen ($P = 0.07$) or liver ($P = 0.3$). The combination, while superior to controls, was not superior to rifampicin alone.

Discussion

The use of rifampicin is a significant problem in highly active antiretroviral therapy (HAART) regimens, which depend on HIV protease inhibitors, because rifampicin
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Accelerates degradation of most protease inhibitors. Azithromycin and clarithromycin have fewer drug interactions than the rifampicin analogues. Rifapentine may cause less induction of hepatic enzymes, and rifabutin even less. Our present studies suggest that azithromycin may be an acceptable substitute for rifampicin in the treatment of disease caused by *M. kansasii*. Azithromycin presumably does not interfere with protease inhibitor therapy of HIV. Rifapentine was chosen because it is a new analogue of rifampicin, with less tendency to accelerate hepatic cytochrome enzyme activity. Rifapentine was also highly effective in prolonging survival, and also at a very low dose of 0.6 mg/kg/day reduced counts when given as monotherapy. Combination therapy has traditionally been used, both to prevent emergence of resistance to *Mycobacterium tuberculosis*, and to shorten duration of therapy. Although we have confirmed the activity of azithromycin, we could not show significant benefit of adding azithromycin to either ethambutol or rifapentine. The doses chosen were selected to optimize potential drug interaction. However, there is a broad interface of doses for potential drug interactions, so the strength of this conclusion is limited to the mouse model, and to the few specific regimens we employed.

References


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