Aspirin and ibuprofen enhance pyrazinamid treatment of murine tuberculosis

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Objectives: Aspirin (acetylsalicylic acid) or ibuprofen [2-(4-isobutyl-phenyl)-propionic acid] was administered to mice undergoing treatment of tuberculosis infection with pyrazinamide to determine if these non-steroidal anti-inflammatory drugs (NSAIDs) enhance pyrazinamide activity in vivo.

Methods: BALB/c mice were infected with Mycobacterium tuberculosis H37Rv through aerosol exposure. Mice were treated orally with aspirin, ibuprofen or pyrazinamide, alone and in combination. The impact of daily administration of these drugs for 1 month was determined by enumerating viable bacteria in the lung and spleen.

Results: Aspirin or ibuprofen alone at 20 mg/kg per day had little effect on tuberculosis infection after 1 month of treatment, while pyrazinamide at 150 mg/kg per day led to a reduction of about 1.5 log10 cfu in the lung and 2 log10 cfu in the spleen compared with the control. Simultaneous administration of either aspirin or ibuprofen with pyrazinamide resulted in a further decrease of about 0.4 log10 cfu in the lung and more than 1 log10 cfu in the spleen compared with mice receiving pyrazinamide alone. All spleens in the pyrazinamid-only treatment group were positive for infection. Of mice treated with both aspirin and pyrazinamide, two of five spleens were negative for colony formation, with a lower limit of detection of 0.90 log10 cfu per organ. Three of five mice given ibuprofen and pyrazinamide had culture-negative spleens.

Conclusions: Aspirin and ibuprofen enhance the effect of pyrazinamide during the initial phase of tuberculosis treatment in the mouse model. Further investigation is necessary to determine the impact of NSAIDs on long-term treatment with pyrazinamide and other antituberculosis drugs in the mouse model of tuberculosis infection and the clinical implications of these findings on tuberculosis treatment in humans.

Keywords: mycobacterium, chemotherapy, salicylate, NSAIDs, tuberculosis

Introduction

Pyrazinamide is a first-line antituberculosis agent that kills non-replicating or slow-growing bacilli under acid pH conditions. When pyrazinamide is given in combination with isoniazid and rifampicin, successful treatment requires at least six months of therapy. New options are needed to shorten the duration of the lengthy therapy. Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin (acetylsalicylic acid) and ibuprofen [2-(4-isobutyl-phenyl)-propionic acid], are commonly used to ameliorate fever and other symptoms of illness. Aspirin belongs to the group of salicylate anti-inflammatory agents. Upon administration, aspirin is rapidly de-acetylated to the active component salicylic acid in the liver of humans and mice. Salicylic acid is thought to reduce prostaglandin synthesis by inhibition of cyclooxygenase, but may also act through separate pathways to elicit the anti-inflammatory effect. Ibuprofen, a derivative of propionic acid, despite its different structure from aspirin, also inhibits the synthesis and release of prostaglandins as mediators of inflammation. In addition to its activity on inflammation, salicylate has been shown in microarray analysis to down-regulate a number of genes involved in transcription, translation and energy production in Mycobacterium tuberculosis. Although previous work in vitro demonstrated that exposure to salicylate reduced

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the susceptibility of *M. tuberculosis* to antibiotics such as isoniazid, streptomycin, rifampicin and ethambutol that act on actively growing bacteria, salicylate had little effect on the susceptibility of *M. tuberculosis* to pyrazinamide in vitro. The inhibition of energy production by salicylate may promote the activity of pyrazinamide, since pyrazinamide is known to deplete membrane energy and energy inhibitors could enhance pyrazinamide activity. Ibuprofen has not been reported to have any effect on *M. tuberculosis* when tested in vitro.

Given that the weak acids benzoic acid and sorbic acid enhance pyrazinamide activity in vitro, and because NSAID use is common and may sometimes overlap with antituberculosis therapy, the current study was designed to examine the effects of aspirin and ibuprofen on pyrazinamide treatment of tuberculosis in the mouse model.

### Materials and methods

**Bacterial strains**

*Mycobacterium tuberculosis* strain H37Rv was obtained from ATCC and cultured in Middlebrook 7H9 liquid medium (Becton Dickinson) with ADC (albumin/dextrose/catalase), 0.2% glycerol and 0.05% Tween 80 (Sigma). To ensure virulence for in vivo studies, strain H37Rv was passaged twice in mice and stored as frozen aliquots. Prior to infection, stocks were thawed and used to prepare fresh subcultures for infection.

**Mouse infections**

Infections were conducted as described previously by Nuermberger and Groset. Four- to six-week-old female BALB/c mice (National Cancer Institute) were infected with aerosolized *M. tuberculosis* H37Rv using a Middlebrook Inhalation Exposure System (Glas-col). Protocols concerning the handling of animals were approved by the Johns Hopkins University Animal Care and Use Committee.

**Treatments**

Following infection, five mice were randomly assigned to each treatment group. Treatment was initiated 1 day after infection and continued 5 days per week for 4 weeks. Drugs were dissolved in sterile water (0.2 mL) and administered by oral gavage. The following dosages were used in the experiments: aspirin (10, 20, 40 mg/kg), ibuprofen (10, 20, 40 mg/kg) and pyrazinamide (150 mg/kg). Compounds were obtained from Sigma. These amounts were chosen for their relevance to the recommended dosages in humans (up to 45 mg/kg or around 3 g per day). For drug combination experiments, 20 mg/kg of aspirin or 20 mg/kg of ibuprofen was given in combination with 150 mg/kg of pyrazinamide. One control group received water only.

**Effect of treatment on colony-forming unit counts in mice**

The day following the conclusion of treatment, mice were euthanized. Total lungs and spleens were homogenized in 2 mL of PBS. Serial dilutions (0.25 mL) of $10^0$, $10^{-2}$, $10^{-4}$ and $10^{-6}$ were plated on Middlebrook 7H11 agar supplemented with ADC and PANTA (Becton Dickinson) to enumerate colony-forming units per total organ per mouse; counts from one group of controls were determined for purposes of comparison.

**Statistical analysis**

Colony-forming unit data were evaluated using Student’s *t*-test.

**Results**

The effect of aspirin or ibuprofen on pyrazinamide activity was evaluated in a murine model of tuberculosis by examining the bacterial counts in the lungs and spleen of the mice following 1 month of therapy.

Control mice after 1 month of infection had $6.9 \pm 0.1$ (standard deviation; SD) $\log_{10}$ cfu in the lung and $4.5 \pm 0.2$ (SD) $\log_{10}$ cfu in the spleen. Pyrazinamide alone reduced counts to $5.6 \pm 0.5$ (SD) $\log_{10}$ cfu in the lung and $2.7 \pm 1.1$ (SD) $\log_{10}$ cfu in the spleen. Administration of ibuprofen alone at 10, 20 and 40 mg/kg had no significant effect on colony-forming unit counts in the lungs or spleen, as compared with controls (Figure 1a and b). High and medium doses of aspirin had no measurable impact on infection, but low-dose aspirin (10 mg/kg) was associated with a small but significant decrease in spleen bacterial counts compared with controls ($P < 0.05$) (Figure 1b). Aspirin treatment had no effect on the colony-forming unit counts in the lungs of these animals.

Aspirin was found to increase the activity of pyrazinamide in the mouse model of tuberculosis treatment (Figure 1c and d). Simultaneous administration of aspirin with pyrazinamide resulted in statistically significant fewer bacteria in the lungs ($5.0 \pm 0.3$ (SD) $\log_{10}$ cfu; $P < 0.05$) than pyrazinamide-only treated groups (Figure 1c). The aspirin and pyrazinamide combination therapy completely cleared the spleen counts in two of five mice, though the other mice remained infected and the decrease seen in the spleens of the animals was not significant compared with pyrazinamide treatment alone ($1.5 \pm 1.5$ (SD) $\log_{10}$ cfu). The lower limit of detection was 0.90 $\log_{10}$ cfu.

Ibuprofen also enhanced the activity of pyrazinamide, much like aspirin, resulting in lower unit counts in the lungs (Figure 1c and d). Again, ibuprofen and pyrazinamide-mediated clearance of infection from spleen was variable ($1.3 \pm 1.6$ (SD) $\log_{10}$ cfu), with no culturable bacteria found in the spleen of three animals while the rest remained positive for infection. The difference in the lungs was more substantial, with lower counts in the ibuprofen and pyrazinamide combination group when compared with the pyrazinamide-only group ($5.1 \pm 0.1$ (SD) $\log_{10}$ cfu; $P < 0.05$).

**Discussion**

We hypothesized that aspirin might enhance the activity of pyrazinamide in vivo, since microarray data suggested that salicylate exposure induces a down-regulation of transcription, translation and energy production, causing a de-energized state in *M. tuberculosis* that is optimal for the activity of pyrazinamide. Indeed, an enhancement of pyrazinamide activity was observed in mice treated with aspirin. Pyrazinamide alone
NSAIDs enhance tuberculosis therapy with pyrazinamide

Figure 1. Amount of bacteria (log_{10} cfu) present in the lung (a, c) and spleen (b, d) following 1 month of treatment of non-established infection with aspirin (ASP), ibuprofen (IBU) or pyrazinamide (PZA). For comparison, controls are presented on the left-hand side of each graph. The three doses of aspirin or ibuprofen (10, 20 and 40 mg/kg) appear in (a) and (b) along with a single dose of pyrazinamide (150 mg/kg). Controls received an equal volume of water. *P < 0.05 as compared with control group; **P < 0.05 as compared with pyrazinamide treated group.

is not completely sterilizing, but with the supplementation of either aspirin or ibuprofen at a clinically relevant dose, additional clearance of viable bacteria was observed in the lungs and spleen. Pyrazinamide has been noted to be effective against non-growing, persistent forms of *M. tuberculosis* by depleting the membrane energy in non-replicating bacilli, so aspirin could be inducing a low metabolic state in the cell that raises its susceptibility to pyrazinamide. It is possible that ibuprofen, as a weak acid, could have a mechanism similar to aspirin’s for increasing pyrazinamide activity. The possibility that these NSAIDs improve the tuberculosis treatment through an effect on host cells, namely inhibiting prostaglandin synthesis and ameliorating inflammation, is less likely, since inhibition of host inflammation by NSAIDs is supposed to antagonize the antibacterial activity of pyrazinamide, which requires acid pH for action. While both compounds have similar effects on inflammation, it is not clear whether ibuprofen could also induce reduced susceptibility to antibiotics in bacteria as does salicylate. Future studies are needed to determine whether ibuprofen can affect drug susceptibility in culture as observed with salicylic acid.

Mice receiving an NSAID along with pyrazinamide, compared with pyrazinamide alone, had modest decreases in lung cfu counts. However, while mice treated with only pyrazinamide did not exhibit spleen cures, the addition of an NSAID led to the complete clearance of culturable bacteria in a portion of spleens. While these results are interesting, a longer TB treatment in mice with NSAIDs in combination with pyrazinamide and other TB drugs is needed to evaluate the role of aspirin and ibuprofen in possible shortening of the therapy and in preventing relapse.

The slightly greater colony-forming unit variability in the pyrazinamide-treated organs (especially the spleens) than other treatment groups (Figure 1) was an outcome that has been observed previously. This phenomenon could be due to the
nature of pyrazinamide, the microenvironment in which the drug is active, or the metabolic status of the bacilli, such that the tuberculocidal effect of pyrazinamide alone can be variable.\(^1\) The discrepancy between the \textit{in vitro} findings that salicylate has no apparent effect on pyrazinamide activity\(^6\) and the \textit{in vivo} observation that salicylate enhances pyrazinamide activity may be due to the inherent difference of the \textit{in vitro} conditions compared with the \textit{in vivo} situation in mice, including the microenvironment and metabolic status of the bacilli.

Although salicylate can induce reduced susceptibility in \textit{M. tuberculosis} to a range of drugs (except pyrazinamide) \textit{in vitro}\(^5\) and also reduced susceptibility to isoniazid in mice,\(^10\) it enhances pyrazinamide activity in mice in this study. It may be possible to take advantage of the enhancement effect of aspirin or ibuprofen on pyrazinamide by administering them separately from other tuberculosis drugs to which salicylate could reduce susceptibility.\(^5,10\) Since the use of aspirin and ibuprofen is prevalent and may overlap with antituberculosis therapy, it is important to determine if any interaction, whether positive or negative, occurs \textit{in vivo} using animal models initially and then in humans.

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\section*{Transparency declarations}

None to declare.

\section*{References}