Ibuprofen Therapy Resulted in Significantly Decreased Tissue Bacillary Loads and Increased Survival in a New Murine Experimental Model of Active Tuberculosis

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(See the editorial commentary by Ivanyi and Zumla on pages 185–8)

C3HeB/FeJ mice infected with Mycobacterium tuberculosis were used in an experimental animal model mimicking active tuberculosis in humans to evaluate the effect of antiinflammatory agents. No other treatment but ibuprofen was given, and it was administered when the animals’ health started to deteriorate. Animals treated with ibuprofen had statistically significant decreases in the size and number of lung lesions, decreases in the bacillary load, and improvements in survival, compared with findings for untreated animals. Because antiinflammatory agents are already on the market, further clinical trials should be done to evaluate this effect in humans as soon as possible, to determine their suitability as coadjuvant tuberculosis treatment.

Keywords. tuberculosis; ibuprofen; active tuberculosis; C3HeB/FeJ mice; bacillary load; survival; tuberculosis treatment; antiinflammatory agents; tuberculosis coadjuvant treatment.

Tuberculosis kills 1.5 million people every year and has tremendous negative health and economic effects, especially in underdeveloped countries [1]. Moreover, the lack of an effective vaccine, the extensive length of treatment, the prevalence of coinfection with human immunodeficiency virus, and the appearance of multidrug-resistant strains worsen the situation [1].

A search for better experimental animal models to study human tuberculosis revealed that the necrotic process in the C3HeB/FeJ mouse strain leads to liquefacted lesions, which resemble those of active tuberculosis in humans. Intravenous infection with a high dose of Mycobacterium tuberculosis H37Rv Pasteur is always followed 3 weeks later by the generation of macroscopically observable lesions, which, on microscopic analysis, show significantly higher neutrophil infiltration when compared to other mouse strains commonly used in tuberculosis research (C57BL/6, BALB/c, DBA/2, 129/Sv, or C3HeN) [2]. These lesions are about 0.5 mm in diameter until day 28, when they suddenly double in size, becoming 4 times greater on day 30 and 8 times greater on day 31. The lesions coalesce and show a center in which the alveolar spaces are invaded by a massive number of cells, mainly neutrophils; progressively become amorphous (caseating necrosis); and continue to evolve toward the destruction of the alveoli cell walls and the softening of the caseum (liquefacted necrosis). Meanwhile, as the lesion sizes excessively increase, mice progressively lose weight and die after a fast deterioration of health (personal communication and unpublished data). This model is very useful because the lesions that develop are very similar to the tuberculosis lesions in immunocompetent human adults before undergoing cavitation [3–5].

Because the massive neutrophil infiltration that characterizes this human-like model suggests the preponderance of the inflammatory response in the evolution of the lesions, we decided to study the effect of antiinflammatory treatment on active tuberculosis.

MATERIALS AND METHODS

A total of 60 C3HeB/FeJ mice were included in the study (the experiment was run 3 times). Six-week-old female C3HeB/FeJ specific-pathogen-free (spf) mice were obtained from Jackson Laboratories (Bar Harbor, ME). The animals were shipped and kept under controlled conditions in a P3 high-security facility with sterile food and water ad libitum. All experimental proceedings were approved and supervised by the Animal Care Committee of the Germans Trias i Pujol University Hospital and by the Department of Environment of the Catalan Government in...
agreement with the European Union Laws for the protection of experimental animals. Mice were supervised daily and euthanized if required following a protocol that monitored weight loss, apparent good health, and behavior, to avoid any suffering. All mice were euthanized with isoflurane (inhalation excess). Two experimental groups were established: the nontreated animals (control group) and the animals treated with ibuprofen (ibuprofen group). Animals were infected intravenously with $2 \times 10^4$ colony-forming units (CFU) of *M. tuberculosis* H37Rv Pasteur.

The ibuprofen group started antiinflammatory treatment at week 3 or 4 after infection, depending on the experiment. To compare the effect of ibuprofen on lesion size and bacillary load in tissue, treatment was started on week 3. To evaluate the effect on the survival, we started treatment 1 week later, on day 28 (week 4), when the animals’ health started deteriorating and would soon compel us to euthanize them, according to the human end-point protocol approved by the ethical committee. We chose to start treatment at this extreme time point during the disease course because we thought this might be similar to the moment when a patient feels really sick, goes to the clinic, and receives a diagnosis of active tuberculosis on the basis of detection of a tuberculous lesion by chest radiography.

We chose ibuprofen because it is a nonsteroidal antiinflammatory drug commonly used as an analgesic and widely distributed. Moreover, ibuprofen is very easy to administer (we used the ibuprofen pediatric syrup Dalsy, 100 mg/5 mL oral suspension, Abbott Laboratories, Abbott Park, IL), which is very important not only for children but also for experiments in mice. Ibuprofen was administered orally at a dose of 80 mg/kg once daily. No other treatments were administered, because we wanted to avoid masking any possible effect of the ibuprofen. A total of 36 animals (18 in each group) were euthanized on day 28, and lungs were extracted to evaluate the effect of ibuprofen after 1 week of treatment. Left lungs were removed, homogenized, and frozen at −80°C until processed.

Figure 1. Macroscopic and microscopic differences between the 2 experimental groups, consisting of controls (A, C, and E) and ibuprofen-treated animals (B, D, and F) 28 days after infection. A and B. Lungs with easily recognizable, white, round-like lesions. C and D. Histologic recuts including lungs of the animals after staining with hematoxylin-eosin. Lesions are stained in dark purple/blue. Treated animals (B and D) showed fewer and smaller lesions than non-treated mice (A and C). E and F. Characteristic center of a lesion in each experimental group. While an amorphous content occupies the intra-alveolar spaces in control mice (E), they are predominantly cellular and filled by neutrophils (mainly apoptotic but also alive) in ibuprofen-treated mice (F).
and plated on 7H11 agar plates. Plates were read after 21 days, and the bacillary load was recorded (in CFU/milliliters). A total of 8 recuts of right lungs per animal were sliced and stained with hematoxylin-eosin for histometric analysis. The area of granulomas relative to the total lung area was analyzed with image analysis software (Matlab vs 7.9.0.529, The MathWorks) in order to obtain the percentage of affected lung area. A total of 12 animals from each group were used to evaluate the effect of ibuprofen on survival.

RESULTS

Oral administration of ibuprofen alone once daily improved the health of the animals. The ibuprofen group showed an impressive reduction in the number and size of the lung lesions, compared with the control group (Figure 1A–D). Histometric analysis confirmed these differences and proved them to be statistically significant (P = .0003, by the Mann–Whitney U test; Figure 2A). Microscopy revealed that the lesions in the control group had an extensive central area characterized by caseous necrosis and liquefactive necrosis, while lesions in the ibuprofen group had intra-alveolar infiltration of neutrophils (both alive and apoptotic) and thickened alveolar walls (Figure 1E and 1F). The treatment also significantly decreased the bacillary load in lungs, as shown in Figure 2B (P < .0001, by the Mann–Whitney U test), and significantly increased survival (Figure 2C). At day 50, about half of the animals treated with ibuprofen were still alive, while <20% of the mice in the control group were alive (P = .0094, by the log-rank test).

DISCUSSION

We demonstrated that ibuprofen alone reduced the percentage of affected lung area, reduced the bacillary load, and increased survival in a mouse model mimicking active tuberculosis in humans.

According to our experience, this experimental animal model has an important inflammatory component that plays a role in the liquefaction of the lesions, based on the massive infiltration of the tissue by neutrophils (personal communication and unpublished data). Because ibuprofen is a nonsteroidal antiinflammatory drug with no activity against M. tuberculosis, as has been demonstrated in vivo in a murine acute infection model [6, 7], its positive effect on human-like lesions in mice with active tuberculosis might be due to its antiinflammatory effect, something that we are now investigating (unpublished data). By decreasing the inflammatory component in the lesions, their evolution toward liquefaction is minimized, and thus the probability that they will resolve is greater.

This effect of ibuprofen suggests that inflammation plays a very important role in the outcome of the tuberculous lesions and, therefore, the disease, something clinically evident if considering the positive effect of adjuvant corticosteroid treatment empirically administered in certain types of tuberculosis (eg, tuberculosis meningitis) [8]. There has been recent interest in understanding the role of inflammation in tuberculosis [9–11]. Research groups worldwide have been studying the effect of aspirin, ibuprofen, and other antiinflammatory agents on a wide range of diseases, with successful results [12, 13], but to our knowledge this is the first study of ibuprofen in the tuberculosis field.

On the assumption that tuberculosis treatment is maximally effective and that the infectious strain of M. tuberculosis is susceptible to antibiotics, at least 6 months will be needed to achieve cure. If the strain is resistant to multiple antibiotics (ie, multidrug resistant [MDR] or extensively drug resistant [XDR]), the treatment length will be increased, with a total duration of 20 months in most patients, as recommended by the World Health Organization, in order to obtain the percentage of affected lung area.

Figure 2. A, Lung histometric analysis. Treated animals showed less affected area over total lung area than nontreated animals. The mean percentages (±SD) obtained after analyzing 8 histological recuts of each animal were 79.85% ± 10.44% for the control group and 20.78% ± 7.94% for the ibuprofen group. Mann–Whitney analysis proved these differences to be statistically significant (P = .0003). B, Effect of ibuprofen on Mycobacterium tuberculosis bacillary load in tissue. Ibufrofen was shown to decrease by about 1 log the bacillary load in lungs when compared to the control group. The SD might be large, but the difference encountered was found to be statistically significant (P < .0001, by the Mann–Whitney U test). C, Effect of ibuprofen on survival. Treating the mice with ibuprofen from day 28 after infection avoided the rapid deterioration in health that the control mice exhibited and significantly increased survival (P = .0094, by the log-rank test).
Health Organization, although complete cure cannot be ensured (the frequency of successful outcomes for patients with MDR-tuberculosis is only 40%-50%) [1]. Moreover, MDR-tuberculosis has implications in terms of costs, as it requires longer and more expensive treatment with second-line drugs, with costs of US$200–800 per year of life saved [1]. So in the best case, treatment will achieve cure in 6 months, and in the worst case no drugs will achieve cure.

On the other hand, the development of a new drug is a very long process. It has to be synthesized, tested in the laboratory, and further tested in several experimental animal models to demonstrate its efficacy and tolerance before undergoing clinical development. Then, several clinical trials (minimum number, 3) are needed, with testing in different human populations. As a result, it can take 10 years for a drug to arrive on the market. The length of the development process and the requirements of the regulatory bodies (whose role is to ensure the safety and efficacy of new pharmaceutical products) make the costs of this process very high [14].

Ibuprofen has already been shown to be safe, even for children. According to the World Health Organization, it is one of the minimum medicines needed in a basic healthcare system, even for children [15]. Moreover, ibuprofen is already on the market, so it does not need to undergo the long development process for new drugs and could be given at present.

For individuals infected with a M. tuberculosis strain nonresistant to drugs, our results indicate that addition of ibuprofen to standard tuberculosis treatment should benefit patients by resulting in a more rapid resolution of the disease. In patients infected with an MDR or XDR strain, the benefit of ibuprofen could even be greater because of its potential use as an adjuvant to the limited therapeutic options currently on offer.

We are aware of the limitations of this pilot study. Ibuprofen plus standard chemotherapy was not tested; thus, we do not know whether the effect of ibuprofen could be even greater. Another antiinflammatory agent, aspirin, was also tested in a low dose by our team and resulted in an increase in survival among mice (data not shown), which suggests that people who regularly consume low-dose aspirin (ie, for cardiac protection) could be protected against tuberculosis.

Our results are encouraging translation toward clinical studies to ascertain the impact of commonly used antiinflammatory drugs in patients with active tuberculosis. Because nonsteroidal antiinflammatory drugs are already available, clinical assays could be planned soon. A simple trial to compare 2 groups of patients with tuberculosis, one receiving standard chemotherapy only and the other receiving standard chemotherapy plus ibuprofen, could be designed. If the effects of ibuprofen we saw in our experimental animal model prove to be half as strong in patients, the consequences of the simple and cheap action of adding ibuprofen as a complement to standard treatment could ameliorate the clinical condition of millions of people, the outcome of their disease, and maybe even save lives.

Notes

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