Introduction

Non-tuberculous mycobacterial infections are very difficult to treat and respond poorly to traditional antituberculous agents. Macrolide antibiotics have emerged in the last decade as the most important drugs for treatment of non-tuberculous mycobacterial infections, including *Mycobacterium avium* complex (MAC) disease, which is the most common systemic bacterial infection in AIDS. Furthermore, interleukin-8 (IL-8) plays an important role in non-tuberculous mycobacterial infections. Increased concentrations of IL-8 were found, for example, in bronchoalveolar lavage fluids from individuals with pulmonary inflammation caused by MAC.

Our preliminary studies showed that administration of azithromycin (a macrolide antibiotic) and clarithromycin (an azalide antibiotic closely related to the macrolide group) to patients with mycobacterial infections increases plasma IL-8 concentrations. Therefore, in the present study we examined the effect of azithromycin and clarithromycin on IL-8 production by human white blood cells. We also explored their effect on human lung macrophages because azithromycin and clarithromycin accumulate primarily in lungs, and because both azithromycin and clarithromycin have been specifically detected in alveolar macrophages.

Materials and methods

**Human subjects**

All studies involving human blood (informed written consent was obtained from all the subjects) and lung macrophages were approved by the Human Subjects Investigation Committee of the University of Texas Health Center at Tyler.

**Reagents**

Azithromycin was provided by Pfizer Inc. (New York, NY, USA) and clarithromycin by Abbott Laboratories (North Chicago, IL, USA). For the stimulation of human white blood cells, the antibiotics were either extracted from tablets with phosphate-buffered saline (PBS) to obtain a 10 mg/mL solution, or pure preparations were dissolved in methanol (4 mg/mL), and then diluted in lipopolysaccharide (LPS)-free PBS (Sigma Chemical Co., St Louis, MO, USA). Alveolar macrophages were stimulated with the pure antibiotic preparations dissolved in methanol.

**Whole blood stimulation**

Blood samples were taken from six healthy donors. Ali-
IL-8 ELISA

The concentration of IL-8 was measured in plasma, red cell lysates and macrophage media in an ELISA assay using a matched antibody pair according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN, USA). Briefly, 96-well microtitre plates were coated with monoclonal anti-human IL-8 antibody. After blocking, the plates were incubated with samples overnight. Then, the plates were washed and incubated with biotinylated goat anti-human IL-8 polyclonal antibody followed by streptavidin–peroxidase (Zymed Laboratories, Inc., San Francisco, CA, USA). The plates were developed with tetramethyl benzidine (Sigma).

Statistical analysis

Differences between groups were analysed by a simple one-way analysis of variance (ANOVA). All statistics were performed using SIGMASTAT (SPSS Science Inc., Chicago, IL, USA).

Results and discussion

When whole blood was stimulated with azithromycin and clarithromycin extracted from tablets, there was a concentration-dependent stimulation of plasma IL-8 production. Azithromycin at concentrations of 0.04, 0.4, 4 and 40 mg/L induced the release of 6879 ± 554, 7549 ± 1319, 8220 ± 1391 and 8890 ± 387 pg/mL of IL-8, respectively (mean ± s.d.) [P < 0.05 compared with control (4208 ± 1568 pg/mL)]. Clarithromycin at the same concentrations triggered the release of 5400 ± 928, 7277 ± 1358, 9153 ± 1394 and 11030 ± 591 pg/mL of IL-8, respectively (mean ± s.d.) [P < 0.05 when compared with control (3524 ± 1568 pg/mL)]. The production of IL-8 associated with red blood cells was also increased although to a significantly lesser extent than plasma IL-8 (P < 0.05). Azithromycin induced the release of 4423 ± 493, 4865 ± 415, 5307 ± 1601 and 5749 ± 728 pg/mL of IL-8, respectively (mean ± s.d.) [P < 0.05 compared with control (1982 ± 466 pg/mL)]. Clarithromycin triggered the release of 3309 ± 1144, 4086 ± 1156, 4863 ± 1123 and 5639 ± 1925 pg/mL of IL-8, respectively (mean ± s.d.) [P < 0.05 when compared with control (1212 ± 466 pg/mL)]. Azithromycin and clarithromycin were less effective in stimulating IL-8 production than LPS (P < 0.05). After LPS stimulation 26112 ± 2351 pg/mL of IL-8 was detected in plasma and 15633 ± 1351 pg/mL of IL-8 was associated with red blood cells (mean ± s.d.).

IL-8 production was increased in a similar manner when the blood was incubated with pure azithromycin and clarithromycin (0.04–40 mg/L) (Figure 1a and b). Both plasma and red blood cell-associated IL-8 were induced as a result of the stimulation (P < 0.05 compared with control) (Figure 1a and b). The amount of IL-8 associated with red blood cells was significantly lower than soluble IL-8 (P < 0.05), and azithromycin and clarithromycin were less effective in stimulating IL-8 production than LPS (P < 0.05) (Figure 1a and b). Appropriate dilutions of methanol alone did not affect IL-8 production.

The macrophages were incubated with azithromycin and clarithromycin (0, 0.4, 4, 40, 400 and 1000 mg/L), which were dissolved in methanol at 5 mg/mL and then diluted in the culture medium. The corresponding dilutions of methanol alone in the culture medium were also added to the cells. To establish whether IL-8 release was affected by cell viability, the cell concentrations were also measured. The effect of different concentrations of the antibiotics and methanol alone on the macrophage viability is shown in Figure 2a. The number of live cells was not affected by methanol alone. There were, however, significantly fewer cells (P < 0.05) detected after treatment with azithromycin or clarithromycin (4, 40, 400 and 1000 mg/L). To account for the differences in cell numbers, the amount of IL-8 released by 10⁷ cells was calculated. The results are summarized in Figure 2(b). Azithromycin at concentrations of 4 and 40 mg/L and clarithromycin at a concentration of 4 mg/L increased IL-8 production (P < 0.05), whereas methanol had no effect. However, either azithromycin at 1000 and 400 mg/L, clarithromycin at 400 mg/L or methanol alone significantly suppressed IL-8 release (P < 0.05)
Macrolide antibiotics alter IL-8 production

(80x313) \( P < 0.05 \) compared with control (0 mg/L). **\( P < 0.05 \) compared with all the concentrations of azithromycin and clarithromycin.

Figure 1. Stimulation of whole blood with several concentrations (0, 0.04, 0.4, 4 and 40 mg/L) of pure azithromycin (■) and clarithromycin (meldrum), and with LPS (10 mg/L; □). (a) IL-8 concentration in plasma. (b) The concentration of IL-8 associated with red blood cells. The data represent the average of three separate experiments with four repetitions in each experiment (± S.D.). *\( P < 0.05 \) compared with control (0 mg/L). **\( P < 0.05 \) compared with all the concentrations of azithromycin and clarithromycin.

Figure 2. Stimulation of human alveolar macrophages with several concentrations (0, 0.4, 4, 40, 400 and 1000 mg/L) of azithromycin (■) and clarithromycin (meldrum), and the corresponding dilutions of methanol (□). (a) The number of viable cells was estimated using MTT. (b) The amount of IL-8 released by \( 10^5 \) cells was calculated. The data represent the average of three separate experiments with four repetitions in each experiment (± S.D.). *\( P < 0.05 \) compared with control (0 mg/L).

In summary, azithromycin and clarithromycin modified IL-8 production by human alveolar macrophages, probably because of their cytotoxic properties.

Stimulation of white blood cells by azithromycin and clarithromycin resulted in increased IL-8 production. This could have a therapeutic significance since Friedland et al. showed that inability to stimulate IL-8 production ex vivo (by whole blood leukocytes) correlated with poor prognosis in patients with tuberculosis. In addition, our report suggests that azithromycin and clarithromycin may affect IL-8 production by human alveolar macrophages. Azithromycin and clarithromycin stimulated production of IL-8 at a concentration of 4 mg/L. Similar levels of these antibiotics are found in lung fluids from volunteers who received azithromycin and clarithromycin. Azithromycin and clarithromycin have been shown to be effective in treating nontuberculous mycobacterial infections, including MAC disease. However, M. avium infection results in delayed inflammatory response, most probably as a result of suppression of synthesis of IL-8 and other cytokines. In this case, release of IL-8 may be beneficial for the host, leading to the accumulation of neutrophils and other inflammatory cells that display mycobactericidal activity. Therefore, azithromycin and clarithromycin may beneficially influence the immunodefence system, especially in immunocompromised individuals. In addition, azithromycin and clarithromycin at a concentration of 1000 mg/L was highly cytotoxic for macrophages. A similar amount of azithromycin and clarithromycin was found in alveolar macrophages obtained from volunteers treated with these antibiotics. Azithromycin and clarithromycin may thus facilitate exposure of intracellular bacteria to neutrophils and other inflammatory cells.

It is important to note that other cell types may also be affected by azithromycin and clarithromycin. Takizawa
et al.,\textsuperscript{10} for example, reported that clarithromycin suppressed IL-8 production by human bronchial epithelial cells but was not cytotoxic for these cells. In summary, our findings indicate that azithromycin and clarithromycin could modify IL-8 function in mycobacterial infections, which may partially explain their efficacy in these infections. Further work is needed to establish what mediators are involved in modulating IL-8 production.

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References


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