Clarithromycin Inhibits NF-κB Activation in Human Peripheral Blood Mononuclear Cells and Pulmonary Epithelial Cells

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Macrolide antibiotics modulate the production of proinflammatory cytokines in vivo and in vitro. Transcription of the genes for these proinflammatory cytokines is regulated by nuclear factor κB (NF-κB). We examined whether or not clarithromycin inhibits the activation of NF-κB induced by tumor necrosis factor alpha (TNF-α) or staphylococcal enterotoxin A (SEA) in human monocytic U-937 cells, a T-cell line (Jurkat), a pulmonary epithelial cell line (A549), and peripheral blood mononuclear cells (PBMC). Flow cytometry revealed that clarithromycin suppresses NF-κB activation induced by TNF-α and Jurkat cells in a concentration-related manner. Western blot analysis also demonstrated that clarithromycin inhibits NF-κB activation induced by TNF-α in U-937 and Jurkat cells and by A549 cells and PBMC and by SEA in PBMC. Western blot analysis of cytoplasmic extracts of A549 cells revealed that this inhibition is not linked to preservation of expression of the IκBα protein. The chloramphenicol acetyltransferase assay indicated that NF-κB-dependent reporter gene expression is suppressed in U-937 cells pretreated with clarithromycin. These findings are consistent with the idea that clarithromycin suppresses the production of proinflammatory cytokines via inhibition of NF-κB activation.

Proinflammatory cytokines are important mediators in inflammation. Macrolide antibiotics exert anti-inflammatory effects through inhibition of the production of proinflammatory cytokines (25, 28, 35, 38, 40, 41). Clarithromycin is a 14-member lactone ring macrolide antibiotic which has been used for the treatment of infectious diseases. It is unclear how clarithromycin suppresses the production of proinflammatory cytokines, but it is not unreasonable to suspect that it inhibits the transcription of multiple cytokine genes.

Nuclear factor κB (NF-κB) is a ubiquitous and important transcription factor for genes that encode proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) (7, 12, 17, 19, 26). The prototype of NF-κB is a heterodimer consisting of p50 and p65 bound by members of the IκB family, including IκBα, in the cytoplasm (2, 3). NF-κB activation requires degradation of the IκB protein (10, 11). Phosphorylation of IκBα by drugs, cytokines, bacterial products, and viruses rapidly leads to IκB degradation and translocation of NF-κB to the nucleus (5, 16). Activation of NF-κB results in the binding of specific promoter elements and expression of mRNAs for proinflammatory cytokine genes (7, 12, 17, 19, 26). We tested the hypothesis that clarithromycin modulates inflammation by inhibiting NF-κB activation in experiments on human monocytic U-937 cells, a T-cell line (Jurkat), a pulmonary epithelial cell line (A549), and peripheral blood mononuclear cells (PBMC) stimulated by TNF-α or staphylococcal enterotoxin A (SEA).

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Flow cytometry of U-937 and Jurkat cells incubated with TNF-α for 30 min demonstrated that clarithromycin inhibited NF-κB activation in a concentration-related manner (Fig. 1). Western blot analysis of nuclear extracts of U-937, Jurkat, and A549 cells stimulated with TNF-α for 2 h revealed that pretreatment with clarithromycin decreased the expression of NF-κB p65 in a concentration-related manner (Fig. 2). Western blot analysis of nuclear extracts of PBMC stimulated with TNF-α or SEA for 2 h demonstrated that pretreatment with clarithromycin also decreased the expression of NF-κB p65 in a concentration-related fashion (Fig. 3).

Inflammation is an important part of the pathogeneses of pulmonary diseases, not only infectious diseases due to bacteria, viruses, and fungi but also chronic obstructive pulmonary disease and neonatal chronic lung disease (32, 37). Clarithromycin inhibits the production of IL-1, IL-6, IL-8, and TNF-α (25, 28). Clarithromycin also modulates antigen-specific T-cell proliferation (25) and improves IL-12-mediated anti-Mycobacterium avium activity (4). How does the clarithromycin action on peripheral blood immunocompetent and pulmonary epithelial cells result in the modulation of inflammation? Clarithromycin must modulate an event or process that is very basic to inflammation. One possibility is that clarithromycin modulates
the transcription of genes for proinflammatory cytokines, the production of which is known to be modulated by clarithromycin.

Our results demonstrate that clarithromycin modulates TNF-α-induced NF-κB activation in U-937, Jurkat, and A549 cells and PBMC and modulates SEA-induced NF-κB activation in PBMC. The results of the CAT assay indicated that clarithromycin inhibits the transcription linked to NF-κB in U-937 cells. It is important to note that, while this report was in the final stage of preparation, Aoki and Kao published evidence consistent with the above observations (1). They noted that Jurkat T cells incubated with erythromycin and stimulated with phorbol 12-myristate 13-acetate and ionomycin showed reduced NF-κB activation. We proved that clarithromycin inhibited NF-κB activation in not only T cells but also monocytes/macrophages and pulmonary epithelial cells.

In infants administered a single oral dose of 5 or 10 mg/kg of body weight, the maximum concentrations of the drug in plasma were 2.26 ± 0.42 and 3.23 μg/ml, respectively (9). In adults administered an oral dose of 500 mg nine times at 12-h intervals, the concentration of clarithromycin in plasma was 3.29 ± 0.94 μg/ml at 4 h (30). The concentrations of clarithromycin in bronchopulmonary epithelial lining fluid (ELF) were 34.02 ± 5.16 μg/ml at 4 h, 20.63 ± 4.49 μg/ml at 8 h, 23.01 ± 11.9 μg/ml at 12 h, and 4.17 ± 0.29 μg/ml at 24 h in adults administered an oral dose of 500 mg nine times at 12-h intervals (30). The mean levels of clarithromycin at a mean time of 4.25 h were 4.0 μg/ml in serum, 20.5 μg/ml in ELF, and 372.7 μg/ml in alveolar cells in adults administered an oral dose of 500 mg seven times at 12-h intervals (13). Our results suggested that therapeutic clarithromycin administration has an anti-inflammatory effect by inhibition of NF-κB activation, because flow-cytometric analysis demonstrated that 3 and 10 μg of clarithromycin/ml significantly inhibited NF-κB activation in U-937 cells and Jurkat cells, respectively. Western blot analysis revealed that only 3 μg of clarithromycin/ml inhibited NF-κB activation in A549 cells.

Western blot analysis indicated that the inhibition of nuclear translocation of NF-κB was not linked to preservation of the IκBα protein. However, several inhibitors of NF-κB activation, such as aspirin, cyclosporin A, IL-10, IL-13, α-melanocyte-stimulating hormone, morphine, estrogen, and pyrrolidine dithiocarbamate, inhibit this translocation by preserving the IκBα protein (15, 18, 20, 22–24, 33, 39, 42). Thus clarithromycin, like IL-4, herbimycin A, and caffeic acid phenethyl ester, suppresses NF-κB activation without interfering with IκBα degradation (6, 21, 29). The precise mechanism underlying the inhibition of NF-κB activation by clarithromycin and these other agents remains unclear. It is possible that clarithromycin inhibits NF-κB activation through modulation of the binding of NF-κB with DNA or by affecting an unknown mechanism in the nuclear translocation of NF-κB.

In summary, our data extend the observation of the anti-inflammatory action of clarithromycin to lung and peripheral blood immunocompetent cells. We conclude that the modulation of NF-κB activation by clarithromycin results in inhibition of the production of proinflammatory cytokines.

REFERENCES


