Immunomodulatory Action of Levofloxacin on Cytokine Production in Adults with Community-Acquired Pneumonia

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Abstract

Background: There is growing evidence that certain antibiotics exert their beneficial effects not only by killing or inhibiting the growth of bacterial pathogens but also indirectly by their immunomodulatory up regulatory effect on the immune system. Immunomodulatory effects of antibiotics could possibly influence the degree of the systemic and local response to infection. So, knowledge of their intrinsic influence on the host’s inflammatory response appears to be essential. Fluoroquinolones have been known to exert modulatory activity on immune responses to microbial infection. However the mechanism of this immunomodulation has not been well elucidated.

Aim of the Work: To explore the Immunomodulatory effect of the frequently used antimicrobial agent, levofloxacin, through evaluating its effect on the serum level of specific cytokine mediators (TNF-α, IL-10) in the pneumonic patients.

Patients and Methods: Serum levels of tumor necrosis factor alpha (TNF-α) and Interleukin-10 (IL-10) have been measured by enzyme linked Immunosorbent assay (ELISA) technique in the sera of 40 patients with community-acquired pneumonia (CAP) before and 10 days after receiving levofloxacin (LVFX), using human TNF-α and IL-10 ELISA kit.

Results: There was a statistically significant decrease in the mean level of TNF-α and a statistically significant increase in the mean level of IL-10 in pneumonic patients after a ten days course of levofloxacin therapy.

Conclusion: This study revealed that treatment with levofloxacin showed significant inhibitory effect of TNF-α as a pro-inflammatory cytokine and a significant stimulatory effect of IL-10 as an anti-inflammatory cytokines which may provide additional benefits in treatment of respiratory tract infections and more efficient eradication of the offending pathogen especially with the emergence of multi-drug resistant microbes. These finding suggested that levofloxacin by its immunomodulatory action on cytokine production may provide additional benefits in patients with chronic pulmonary infections that are independent of its antibacterial properties.

Key Words: Levofloxacin – TNF-α – IL-10 – Immunomodulatory effect – Fluoroquinolones.

Introduction

There is growing evidence that certain antibiotics exert their beneficial effects not only by killing or inhibiting the growth of bacterial pathogens but also indirectly by their immunomodulatory effects on the immune system. For more than 15 years, it has been noted that certain antibiotics like macrolides and fluoroquinolones have immunomodulatory properties that improve the long term outcome of patients with chronic inflammatory pulmonary diseases [1].

Levofloxacin (LVFX) is one of the newest third generation fluoroquinolones, it is the bacteriologically active L-isomer of ofloxacin. Levofloxacin has a broad spectrum of action, it diffuses through bacterial cell wall and acts by inhibiting and disrupting the function of DNA gyrase (bacterial type II topoisomerases) which is an enzyme that regulates the over winding or unwinding of DNA and is required for DNA replication, repair and RNA transcription. This inhibition of DNA gyrase activity leads to blockage of bacterial cell growth. So LVFX acts as efficient anti-bacterial by hijacking the natural ability of topoisomerase to create breaks in chromosomal DNA [2].

Levofloxacin is a highly appropriate agent for treating respiratory tract infections because it has a broad spectrum anti-bacterial action for all of the most common respiratory tract infection pathogens, being effective against Gram-negative and Gram-positive, as well as atypical organisms. Also, due to its excellent pharmacokinetic and pharmacodynamic features which allow it to penetrate extremely well into lung tissue and bronchial...
secretions. In addition it has the ability to penetrate into both phagocytic and epithelial cells which
appears to be extremely important in inhibition of intracellular organisms. So, LVFX achieves high
concentrations in respiratory secretion and lung tissue and has persistent activity in lung tissue “post antibiotic effect” (PAE) [3].

In addition to its antibacterial activity, LVFX has immunomodulatory effects that are independent of its antibacterial properties. The molecular mechanisms causing immunomodulatory effect of LVFX are still under investigations. However, very recently, activation of p38 mitogen-activated protein kinase (MAPK) pathway, which is considered one of the major signal transduction pathway involved in inflammatory responses, was proposed as the main effect of LVFX [1].

Many studies were done to evaluate immunomodulatory effect of LVFX on pro-inflammatory cytokine production and it showed that LVFX has a dose-related reduction of some pro-inflammatory cytokine secretion. LVFX can suppress production of interleukin-1β (IL-1β) by Lipopolysaccharide-stimulated (LPS-PBMC) stimulated peripheral blood mononuclear cells in a concentration dependent manner. LVFX can suppress TNF-α production at only highest concentration. LVFX increase (interleukin-2) IL-2 production by (PBMC) stimulated with phytohemagglutinin (PHA) in a dose-dependent manner. The (Granulocyte Macrophage-Colony stimulating Factor) GM-CSF and soluble IL-2 receptor production by PHA-stimulated (PBMC) was suppressed at high concentrations of LVFX [4].

Fluoroquinolones were found to be effective in vivo either in infection caused by organisms against which these are inactive or when dosed sub optimally. These in vivo effects were correlated with a significant decrease in pro-inflammatory cytokines like (interleukin-1) IL-1 and TNF so they have a pronounced effect on cytokine expression which is the key regulators of the inflammatory response to infection and main stimulants of chemotaxis of immune cells to sites of infection [5].

Several general observations explain the various immunomodulating effects of quinolones in eukaryotic cells. Firstly, effect of quinolones on cyclic adenosine monophosphate (cAMP), protein Kinase A (PKA), and phosphodiesterases (PDE). Secondly, effects of quinolones on signal Transduction and critical intracellular Transcription Factors such as (nuclear factor kappa beta) NF-κB, Ap-1, NF-II-6, NFAT which affect the level of mRNA of specific cytokines and also cytokine gene expression and transcription. Lastly, the role of “quinolones topoisomerase II interactions” leading to an eukaryotic equivalent of bacterial SOS response. The bacterial SOS response is characterized by a series of reactions affecting various transcription factors and kinases aimed at inhibiting cell division and enhancing DNA repair in an attempt to prevent the cell death. Thus, the immunomodulating effects exerted by quinolones are the consequence of inhibition of topoisomerase II by Fluoroquinolones leading to a mammalian stress response that bears similarities to the bacterial SOS response exerted by quinolones [6].

Aim of the work:

This study aimed to evaluate the immunomodulatory effect of Levofloxacin through its effect on pro-inflammatory cytokine production either (stimulation or suppression) of (TNF-α, IL-10) in pneumonic patients to identify potential means of improving the host response to pathogens by selective augmentation or depletion of specific cytokine mediators to more efficiently eradicate the offending pathogen especially with the emergence of multi-drug resistant microbes and an increase in the population at risk for development of pneumonia.

Patients and Methods

Patient selection and treatment:

This prospective study was conducted among adult patients with community-acquired pneumonia from April 2011 to June 2013. The study included forty adult patients with confirmed diagnosis as pneumonia, with age ranged from 42-67 years, 28 males and 12 females, attending to Assiut University hospital outpatient fever clinic and those admitted to infectious disease unit, Tropical Medicine Department. Cytokine assay was performed (by measuring TNF-α and IL-10 in serum samples) in the Departments of Medical Microbiology and Immunology Laboratory, Faculty of Medicine, Assiut University. Blood samples were taken before and 10 days after receiving course of Levofloxacin antibiotic (750mg once daily). Intravenous therapy was maintained during the first 5 days of admission and was switched to oral therapy afterward. Local research ethics committee was approved and informed consent was taken from all patients.

Inclusion criteria:

Adults, males and females with confirmed pneumonia were included in the study. Pneumonia is defined as an acute febrile respiratory illness accompanied by a new radiographic shadow consistent with this diagnosis.
**Exclusion criteria:**

Age <18 years, pulmonary tuberculosis, bronchiectasis, known allergy to fluoroquinolones, underlying systemic autoimmune disease, and immunocompromised states, including patients on maintenance oral corticosteroids, pregnant females, patients who received antimicrobial therapy in the 15 days preceding the actual episode, and patients who had received fluoroquinolones in the last month or who had received nonsteroidal anti-inflammatory therapy in the last 2 weeks. Patients with serum creatinine at 2mg/dl or patients on hemodialysis, as well as patients with documented pneumococcal pneumonia within the previous 4 weeks, were also excluded.

On admission, data were prospectively collected and included demographic characteristics. All patients were subjected to complete clinical assessment. Routine investigations were performed including: complete blood count, X-ray chest, and sputum examination. Patients with confirmed diagnosis as having pneumonia were included in the study. Samples were obtained in sterile tubes without any anti-coagulant; each tube was labeled with the patient’s name, sex, age and the date of collection. Samples were spun down at 2000rpm for 10 minutes, the serum was stored in an epindorphe tube which was labeled appropriately and stored at 20°C.

Cytokine assay was performed by measuring TNF-α and IL-10 in serum samples using human TNF-α and IL-10 ELISA kit Koma biotech respectively. The Koma biotech human cytokines ELISA kit is an enzyme linked immunosorbent assay for quantitative measurement of human cytokines in the serum. All tests were done according to the manufacturer’s instructions through the following steps:

- 200ul of washing solution were added to each well then the wells were aspirated to remove liquid and the plate washed 3 times using 300ul of washing solution per well.
- 100ul of sample were added to each well. The plate were covered with the plate sealer and incubated at room temperature for 2 hours.
- The wells were aspirated to remove liquid and the plate washed 4 times like as step 1.
- 100ul of the diluted detection antibody (0.5ug/ml) were added per well, the plate covered with the plate sealer and incubated at room temperate for 2 hours.
- The wells were aspirated to remove liquid and the plate washed 4 times like as step 1.
- 100ul of the diluted color development Enzyme (1:20 dilute) were added per well, the plate covered with the plate sealer and incubated 30 minutes at room temperate.
- The wells were aspirated to remove liquid and the plate washed 4 times like as step 1.
- 100ul of color development solution were added to each well. The plate were incubated at room temperature for a proper color development (8-18 minutes), 100ul of stop solution were added to each well to stop the color reaction.
- The micro plate reader wave length was set at 450nm and the absorbance (OD) of each well was measured.

**Statistical analysis:**

All data were analyzed using the computerized statistical analysis (Statistical package for social science “SPSS, version 16”) concentrations of TNF-α and IL-10 were expressed as Mean ± Standard deviation. Differences in mean values of TNF-α concentrations and IL-10 concentrations before and after receiving levofloxacin were calculated using Wilcoxon Signed Ranks Test. *p*-value is significant when less than 0.05.

**Results**

Levofloxacin caused a statistically significant decrease in the mean level of TNF-α in the studied patients when we compared levels before and after therapy (*p*<0.05) in Table (1) and Fig. (1). According to the CDC the normal reference range of TNF-α is less than 10pg/ml. The mean value of TNF-α in patient before taking LV was 36.43±4.18pg/ml was and decreased to 20.82±1.31pg/ml after 10 days LVFX therapy (*p* =0.004).

Regarding IL-10, LVFX caused a statistically significant increase in the mean level of IL-10 in patients. According to the CDC the normal reference range of IL-10 is less than 1.25-15.66pg/ml. Here, the mean value of IL-10 in patient before receiving LVFX was 42.54±2.83pg/ml and increase to 61.75±2.85pg/ml after 10 days of therapy which was statistically significant (*p* =0.001).

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<th>Before treatment</th>
<th>After treatment</th>
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<tr>
<td><strong>TNF-α</strong></td>
<td>36.43±4.18</td>
<td>20.82±1.31</td>
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<td><strong>IL-10</strong></td>
<td>42.54±2.83</td>
<td>61.75±2.85</td>
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Table (1): Mean values of serum TNF-α and IL-10 in the studied patients with pneumonia.
Discussion

Immunomodulatory drugs may enhance host defense by either inhibiting inflammatory response or induction of counter-regulatory anti-inflammatory mechanism [6].

Several classes of antibiotics, including macrolides and quinolones exert modulatory effects on cytokine release by inflammatory cells [7].

It is important to define the Immunomodulatory effects of these antibiotics which are commonly used in the therapy of respiratory tract infections, these effects seem to be related to the bacterial killing as well as the resolution of local inflammation, this may account for the therapeutic benefit of LVFX in these type of infections, even when bacterial eradication is not complete [8].

There is a general trend that fluoroquinolones decrease the synthesis of pro-inflammatory cytokines. All the fluoroquinolones studied exerted significant clinical effects by attenuating cytokine responses in vivo [9].

The fluoroquinolones were found to be effective in vivo either in infections caused by organisms against which these are inactive or when dosed suboptimally. These in vivo effects were correlated with a significant decrease in pro-inflammatory cytokines like IL-1 and TNF [5].

The aim of this work was to evaluate the action of LVFX on cytokines in pneumonic patients in order to clarify its role in modification of the outcome of respiratory tract infection.

Recent studies have demonstrated that in the early stages of infection, the local generation of pro-inflammatory cytokines such as TNF-α, IL-β, IL-8, IL-12, IFN-γ and possibly IL-6 are triggered by bacterial infection [10].

In the present study we found that levofloxacin led to statistically significant decrease in the mean level of TNF-α in the studied patients, which is consistent with the results obtained by Yoshimura et al., 1996, who found that levofloxacin suppressed tumor necrosis factor production by peripheral mononuclear cells (PBMC) at only the highest concentration [4].

Also, Calbo et al., showed that the level of TNF-α as well as other pro-inflammatory cytokines reduced in pneumonic patients treated with levofloxacin [11].

Tumor necrosis factor is by far the best studied in pulmonary host defense, and has been shown to be of critical importance in a variety of animal models of pneumonia and a central mediator of the host’s response to infection. It is rapidly produced following either antigen-specific or nonspecific stimulation and has, therefore, been designate an early response, or “alarm”, cytokine [12].

Lipopolysaccharide (LPS) is the best studied and most potent stimulus for TNF production. In Gram-negative bacteria, LPS is the major pro-inflammatory component of the cell walls, and the study of LPS-induced TNF expression by alveolar macrophages is, accordingly, very relevant to the role of TNF in the host defense response during Gram-negative pneumonia [13].

TNF is predominantly produced by cells of myeloid lineage, and serves as a major activator of both neutrophils and macrophages. Specifically, TNF enhances leukocyte microbial killing by augmenting phagocytosis, oxidative burst, and release of proteases [14].

TNF also contributes to the accumulation of neutrophils in the area of inflammation by stimulating the expression of adhesion molecules on both vascular endothelial cells and phagocytic cells, and by inducing the production of chemotactic cytokines [15].

Increased expression of TNF in the lungs has been observed in patients with bacterial pneumonia [16].

Many mechanisms had been described to explain these findings; First, the ability of levofloxacin to inhibit the production of TNF-α, which occurs in very early stages of its synthesis, probably due to its effect as a phosphodiesterase inhibitor, leading to cyclic AMP accumulation in the cells, resulting in enhanced cyclic AMP-protein kinase A activity, which in turn is known to inhibit TNF-α production [17]. Others have described the ability of fluoroquinolones, such as levofloxacin, to interfere with
NF-κB activation by inhibiting the degradation of IkBα, thus reducing the levels of production of pro-inflammatory cytokines \[18\].

So levofloxacin by its inhibitory effect on TNF-α may complement its direct antibacterial action by enhancing cellular defense mechanisms and facilitate the resolution of undesirably prolonged lung inflammation and improve outcome of infection \[4\].

IL-10 is a cytokine with potent inhibitory effects on T-1 T cells and antigen presenting cells, such as monocyte/macrophages, causing down regulation of expression of major histocompatibility complex (MHC) class II molecules and attenuated release of pro-inflammatory cytokines, including TNF, T-1 phenotype cytokines IFN-γ and IL-12, and members of both C-X-C and C-C families of cytokines \[19\].

In our study, we found that serum levels of IL-10 were significantly elevated in all patients after taking levofloxacin, which agree with the results obtained by Calbo et al., 2008 \[11\].

It is well known that excessive production of pro-inflammatory cytokine mediators can induce systemic inflammatory response syndrome and that these cytokines play an important role in the development of acute respiratory distress syndrome and multiple-organ dysfunction \[20\]. IL-10 as an anti-inflammatory cytokines acts as specific inhibitor of this network \[21\].

**Conclusion:**

Our study revealed that treatment with the fluoroquinolone agent, levofloxacin, affects production of TNF-α as a pro-inflammatory cytokine and IL-10 as an anti-inflammatory cytokines which may provide additional benefits in treatment of respiratory tract infections to more efficiently eradicate the offending pathogen especially with the emergence of multi-drug resistant microbes and an increase in the population at risk for development of pneumonia. So, LIVX, by its suppressive effect on the production of TNF-α and stimulatory effect of production of IL-10 achieve the balance between pro and counter inflammatory agents which determine the final outcome of infection and clinical course of the disease.

**References**


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