Clarithromycin: A Promising Immunomodulator in Sepsis

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III

Introduction

Severe sepsis and septic shock are among the leading causes of death, representing the 10th most common cause of death in the United States of America [1]. The high mortality rates, ranging between 35 and 50 % despite adequate antimicrobial treatment [2], have encouraged intense research efforts to better understand the mechanisms underlying the pathogenesis of sepsis. As a consequence, sepsis syndrome is now recognized as a complex entity created by an intense inflammatory reaction that is generated in the host after stimulation of the innate and adaptive immune systems by bacterial components [3].

Understanding that sepsis is a hyper-inflammatory reaction of the host triggered by invading bacteria created the need for therapies aimed at modulating the exaggerated host response. Numerous experimental and clinical studies have been published in this field. Anti-endotoxin antibodies, anti-tumor necrosis factor (TNF)-α antibodies, soluble TNF-α receptors, recombinant human activated protein C (rhAPC), low dose hydrocortisone, and intensive insulin therapy are just some of the compounds that have been proposed. Clinical trials with most of these agents have failed to disclose any clinical benefit or have shown limited clinical efficacy. Published guidelines by the Surviving Sepsis Campaign [4] have restricted the application of immunotherapy to only three arms: a) Administration of rhAPC with a 2B grade of evidence in patients with an APACHE II> 25; b) low dose hydrocortisone with a 2C grade of evidence in patients with septic shock; and c) tight glucose monitoring to maintain glucose concentrations below 150 mg/dl with a 2C grade of evidence in patients with severe sepsis and septic shock. The above mentioned low grades of evidence, often resulting from the serious adverse effects of the suggested immunotherapies, underline the need for the evolution of new strategies of immuno-intervention with greater clinical efficacy and without serious adverse events.

The present chapter analyzes the evolution of intravenously administered clarithromycin as an immunomodulator in sepsis. The chapter is organized into three parts: a) Evidence from observational studies about promising anti-inflammatory effects of macrolides in pneumonia; b) presentation of the effect of clarithromycin in experimental studies of sepsis; and c) analysis of results from one recent randomized trial showing considerable clinical efficacy of clarithromycin in patients with ventilator-associated pneumonia (VAP) and sepsis.

Indirect Evidence for an Immunomodulatory effect of Macrolides in Pneumonia

Macrolides have been shown to be effective in chronic inflammatory disorders of the lower respiratory tract, namely diffuse panbronchiolitis and cystic fibrosis [5]. Diffuse panbronchiolitis is a chronic obstructive disease of the airways leading to early death due to respiratory failure and cor pulmonale. Survival has been considerably prolonged after introduction of erythromycin into the daily treatment of these patients in 1979. Daily oral administration of 500 mg of clarithromycin is the treatment of choice nowadays. Four randomized clinical trials have been published in patients with cystic fibrosis. In all these trials, enrolled patients were allocated to either placebo or azithromycin. Administration of azithromycin was accompanied by improvement of respiratory function, as shown by an increase in the forced expiratory volume in one second (FEV_1) and by a considerable reduction in exacerbations of the disease [6–9].

In all the above studies, the proposed mechanism of action of macrolides may involve either a direct effect on *Pseudomonas aeruginosa* colonizing the airways of the patients or an effect on the immune system of the host [5]. This mechanism of action is difficult to demonstrate in acute inflammation of the airways, namely in pneumonia, and no randomized trial has ever been conducted to provide such evidence. As a consequence, only indirect evidence is available, coming from retrospective observational studies. Results of these studies are summarized in **Table 1** [10–14]. A common denominator of these studies is the positive effect of the administration of a macrolide on patient outcome. Addition of a macrolide to a β-lactam was consistently accompanied by a considerable reduction in mortality. This was particularly pronounced when pneumonia was aggravated by bacteremia or severe sepsis. One probable explanation for the clinical benefit seen with macrolides could be their effect against atypical pathogens. However, even when the analysis included only patients infected by *Streptococcus pneumoniae*, the macrolide benefit was still apparent [10, 11]. The only evidence opposing a beneficial effect of macrolides in patients with pneumococcal pneumonia comes from analysis of a prospective cohort

Table 1. Summary of retrospective observational trials providing indirect evidence for an immunomodulatory effect of macrolides in pneumonia.

1st author [ref]	Number of patients	Effect of macrolide
Martinez [10]	409 patients with pneumococcal bacteremia	Addition of a macrolide to a β-lactam reduced the relative risk for death 2.5-fold
García Vázquez [11]	1391 patients with CAP	Therapy with β-lactam+ macrolide reduced mortality (6.9 %) compared to monotherapy with β-lactam (13.3 %)
Lodise [12]	2349 episodes of CAP and bacteremia	Independent factor connected with: ↓ in-hospital mortality, ↓ 30-day mortality
Metersky [13]	1560 patients with CAP	Therapy with β-lactam+ macrolide reduced mortality (18.4 %) than monotherapy with fluoroquinolone (36.6 %)
Restrepo [14]	237 patients with CAP and severe sepsis	Addition of a macrolide to antimicrobials decreased mortality in patients with macrolide-resistant pathogens

CAP: community-acquired pneumonia; ↓: reduction

of 638 Spanish patients. In these patients, addition of a macrolide to a β-lactam did not have any influence on mortality [15].

Lessons from Animal Studies

Clarithromycin was chosen as the most promising candidate among the macrolides for immunomodulation in sepsis. Selection was based on its *in vitro* efficacy and on its pharmacokinetics. *In vitro* studies showed that clarithromycin inhibited the production of interleukin (IL)-8 by both human monocytes and by monocytes of the THP-1 human leukemia cell line after stimulation with cell lysates of *P. aeruginosa* and of *Escherichia coli*. The effect of clarithromycin was dose-dependent and was greater at concentrations closer to 10 μg/ml in the growth medium; the effect was mediated through inhibition of nuclear factor-kappa B (NF-κB) [16]. Concentrations of clarithromycin in the epithelial lining fluid, which is the site of invading microorganisms in pneumonia, after oral administration range between 15 and 70 μg/ml [17]; those of azithromycin are equal to 1 μg/ml [18]. The need for concentrations close to 10 μg/ml to inhibit IL-8 production by monocytes, which are only achieved in the epithelial lining fluid by clarithromycin, led to its selection for further animal studies.

The efficacy of intravenously administered clarithromycin was tested in a series of animal studies [19–24]. Experimental sepsis was induced in rabbits by a model of complicated acute pyelonephritis closely resembling the human situation. In that model, the upper part of the ureter was ligated close to the renal pelvis and the offending pathogen was inoculated above the ligation inside the pelvis. Bacterial challenge was induced by bloodstream isolates from patients with severe sepsis. These isolates were antimicrobial-susceptible *E. coli*, multidrug-resistant *P. aeruginosa*, and pandrug-resistant *Klebsiella pneumoniae*. Clarithromycin did not affect *in vitro* bacterial growth of the selected isolates in time-kill assays. It was administered to animals either in parallel with bacterial inoculation or after bacterial challenge and upon presentation of signs of sepsis. These time windows for the administration of clarithromycin were selected in order to evaluate its efficacy in a model of late sepsis and to avoid past mistakes in which proposed immunomodulators were proven effective as pre-treatment but ineffective in clinical trials [25].

Clarithromycin was administered as either two consecutive intravenous doses for just one day or as one daily dose for three consecutive days. The rationale of dosing was to achieve serum levels close to 10 μg/ml. In all experiments, a single dose of amikacin was administered either alone or with clarithromycin. This was done in an attempt to simulate clinical practice where some antimicrobials are prescribed even for infections by multidrug-resistant pathogens. For infections by susceptible *E. coli* and multidrug-resistant *P. aeruginosa*, survival was the primary end-point. For infections by pandrug-resistant *K. pneumoniae*, animals were sacrificed at standard time intervals to assess tissue histopathology. Concentrations of endotoxins, TNF-α and malondialdehyde (MDA) were estimated in serum at serial time intervals. Blood monocytes were also isolated and assessed for their *ex vivo* release of TNF-α and for the intracellular activity of caspase-3.

Results from these animal studies [19–24] revealed that clarithromycin, either alone or in co-administration with amikacin, prolonged survival considerably. This was accompanied by improvement in oxygen saturation and heart rate. Although all animals had the same degree of endotoxemia and thus the same risk of developing

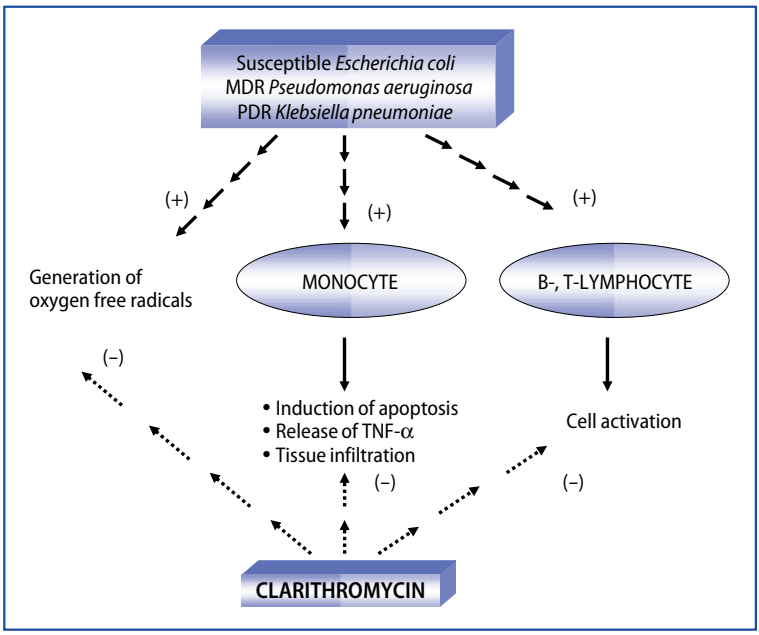

Fig. 1. Proposed mechanisms of action of clarithromycin based on experimental studies in sepsis. MDR: multidrug-resistant; PDR: pandrug-resistant; TNF: tumor necrosis factor; (+): activation; (–): inhibition

a septic reaction, those treated with clarithromycin had lower serum levels of TNF-α and MDA. This finding suggested attenuation of the systemic inflammatory response and of the generation of oxygen free radicals. Clarithromycin did not affect tissue growth of the test isolates. Pathology scores for the kidney, liver, lung and spleen were, however, lower among clarithromycin-treated animals than controls. More precisely, clarithromycin attenuated: a) peribronchial inflammation in the lung; b) mononuclear infliltration and necrosis in the liver and kidney; and c) activation of B- and T-cell rich areas in spleen. The effect of clarithromycin was most notable on the function of monocytes. Induction of apoptosis was attenuated as evidenced by a decrease in the intracellular activity of caspase-3; *ex vivo* release of TNF-α was also decreased.

The goal of the treatment regimen was achieved since serum levels of clarithromycin within two hours after the end of the infusion ranged between 5 and 10 μg/ml. The mode of action of clarithromycin, based on knowledge derived from animal studies, is summarized in **Figure 1**. Monocytes and lymphocytes appear to be the most likely cell targets of clarithromycin due to amelioration of the function of monocytes and to the reduction of tissue infiltration by mononuclear cells observed in animal studies.

Clinical Efficacy of Clarithromycin as an Immunomodulator in Sepsis

The promising results of experimental studies led us to design a randomized clinical trial of the immunomodulatory effect of clarithromycin in patients with sepsis. It is postulated that part of the failure of previous clinical trials with immunomodulators

was due to the inclusion of heterogenous groups of patients, namely patients with sepsis caused by different types of infections [25]. In our trial, all patients had the same underlying infection causing sepsis, namely VAP. A total of 200 patients were enrolled in a prospective double-blind, placebo-controlled, randomized trial over the period June 2004-November 2005. Patients were allocated to either placebo or clarithromycin. One gram of clarithromycin diluted in 250 ml of 5 % glucose was infused within one hour through a central catheter once daily. This regimen was expected to provide serum levels of clarithromycin within those required to demonstrate its immunomodulatory properties, as assessed by preliminary pharmacokinetic studies [26]. The administered antimicrobials were selected by the attending physicians. Primary end-points were sepsis-related mortality, progression to multiple-organ dysfunction and resolution of VAP [27].

One hundred patients received placebo and another 100 patients received clarithromycin. There were no differences between groups in baseline characteristics, namely age, sex, APACHE II scores, and number of failing organs. All patients were screened for the underlying pathogen by quantitative cultures of tracheobronchial secretions. Cultures yielding a pathogen at a count $\geq 1 \times 10^6$ colony forming units (cfu)/ml were considered positive. There were no differences between the groups in the types of causative pathogens. Gram-negative bacteria alone were identified as underlying pathogens in 68 placebo-treated and 66 clarithromycin-treated patients. The most frequent pathogens were *Acinetobacter baumannii* in 43 and 36 patients, respectively, and *P. aeruginosa* in 12 and 17 patients, respectively. Based on the antibiograms of the pathogens, initial empirical antimicrobial coverage was active against 62.7 % of pathogens isolated from placebo-treated patients and against 75.4 % of pathogens isolated from clarithromycin-treated patients ($p = 0.44$ between groups). Tracheobronchial secretions were sampled again at follow-up. Eradication of the pathogen was achieved in 25.4 % and 33.8 % of cases, respectively, on day 5 ($p = 0.31$ between groups) and in 31.3 % and 29.2 % of cases, respectively, on day 10 ($p = 0.82$ between groups).

Sepsis-related mortality was 25 % in the placebo group and 23.3 % in the clarithromycin group. Odds ratio (OR) for death from septic shock and multiple organ failure was 19.00 (95 % confidence intervals: 5.64 – 64.03) in placebo-treated patients. It was reduced to 3.78 (95 % confidence intervals: 1.36 – 10.45) in clarithromycin-treated patients ($p = 0.043$ between groups). VAP resolved in 72.2 % of survivors treated with placebo and in 79.9 % of survivors treated with clarithromycin. Median time to resolution of VAP was 15.5 days in the placebo group and 10.0 days in the clarithromycin group. Comparative cumulative curves of the time to resolution of VAP for each treatment arm are shown in **Figure 2**. The mean clinical pulmonary infection scores (CPIS) for the placebo and the clarithromycin group on study enrolment were 7.92 and 7.62, respectively ($p = 0.29$). These decreased to 6.10 and 5.23, respectively, on day 5 of follow-up ($p = 0.016$) and to 5.88 and 5.09, respectively, on day 10 of follow-up ($p = 0.032$).

Weaning from mechanical ventilation was performed in 58.6 % of placebo-treated patients within a median period of 22.5 days, and in 72.5 % of clarithromycin-treated patients within a median period of 16.0 days. Comparative cumulative curves of the time to weaning for each treatment arm are shown in **Figure 3**. Eight and 14 patients of the placebo and clarithromycin groups, respectively, progressed to develop multiple organ failure. The mean time to progression to multiple organ failure was 3.38 and 5.78 days, respectively ($p = 0.047$ between groups). The two groups did not differ regarding the occurrence of serious adverse events.

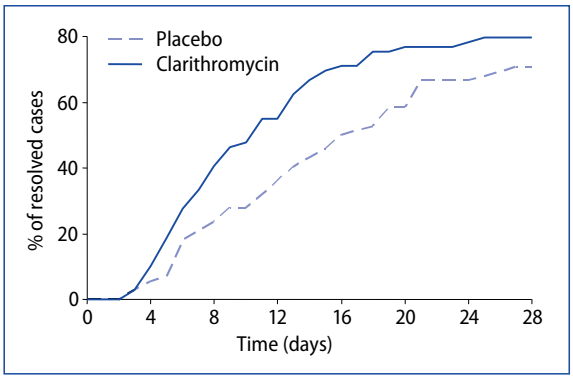

Fig. 2. Cumulative incidence of the resolution of ventilator-associated pneumonia (VAP) within the follow-up period of 28 days ($p = 0.011$ between groups). Analysis comprised survivors. From [27] with permission.

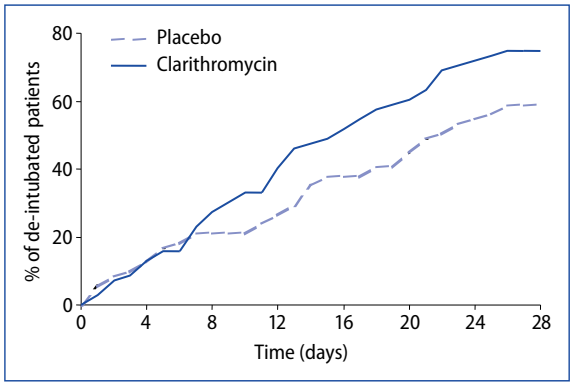

Fig. 3. Cumulative time to weaning from mechanical ventilation among placebo- and clarithromycin-treated patients ($p = 0.049$ between groups). Analysis comprised survivors. From [27] with permission.

The analysis of the randomized clinical trial [27] clearly showed that administration of clarithromycin was beneficial for the patients. This benefit was related to an improvement in the underlying infection and to an effect on the septic mechanism. The beneficial effect of clarithromycin in VAP was shown by: a) earlier resolution of VAP; and b) earlier weaning from mechanical ventilation. The effect of clarithromycin on the septic mechanism is supported by: a) the above mentioned similarities of both groups regarding disease severity and adequacy of antimicrobial therapy; b) the reduction in the relative risk of death due to septic shock and multiple organ dysfunction; and b) the prolongation of time to progression to multiple organ dysfunction.

Conclusion

Results of the trial discussed above [27] are very encouraging since statistical benefit was apparent with only 100 patients enrolled per treatment arm. However, to fully elucidate the future role of clarithromycin as an immunomodulator, further investigation is necessary. A second randomized trial in 600 patients with microbiologically or clinically documented sepsis by Gram-negative bacteria started in July 2007. This study is being conducted in six centers in Greece after approval by the National Ethics Committee (No 76306/13.06.2007) and the National Organization for Medi-

cines (No 76305/15.02.2007). Enrolled patients have sepsis and primary or secondary Gram-negative bacteremia or intrabdominal infection or acute pyelonephritis. Clarithromycin seems a promising and safe new strategy for immunointervention in sepsis. The ongoing trial may verify its clinical efficacy.

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