Immunomodulatory therapies for sepsis: unexpected effects with macrolides

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Despite intensive efforts to increase our knowledge of the inflammatory pathways involved in the pathogenesis of sepsis, several clinical trials of agents aimed at modulating the immune response of the host, such as anti-endotoxin antibodies, anti-tumour necrosis factor (TNF) antibodies and soluble TNF receptors, have failed to disclose any definite clinical benefit. The same applies to the administration of low-dose hydrocortisone as well as intense glucose control by continuous insulin infusion. Macrolides are a traditional class of antimicrobials proven to act as modulators of the host's response in chronic lung disorders such as diffuse panbronchiolitis and cystic fibrosis. The favourable outcome of community-acquired pneumonia treated with the combination of a β-lactam and a macrolide is partly attributed to their immunomodulatory properties. Based on favourable responses to intravenous administration of clarithromycin in experimental models of sepsis by susceptible and multidrug-resistant Gram-negative isolates, a randomised clinical trial was conducted in 200 patients with ventilator-associated pneumonia (VAP) and sepsis (http://www.clinicaltrials.gov; NCT00297674). Clarithromycin was administered at a dose of 1 g within 1 h of infusion for three consecutive days. Analysis revealed a considerable benefit of clarithromycin in shortening the time to resolution of VAP and to de-intubation from mechanical ventilation. The relative risk of death by septic shock and multiple organ dysfunction was 19.00 among placebo-treated patients; it was reduced to 3.78 among clarithromycin-treated patients. These results render new perspectives for the future application of clarithromycin as an immunomodulatory therapy of sepsis.

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1. **Introduction**

Sepsis is defined as the fifth leading cause of death in the USA [1]. A recent survey in Germany disclosed a 12% overall prevalence of sepsis among patients hospitalised in Intensive Care Units (ICU); the respective prevalence rate for severe sepsis was 11%. The ICU and hospital mortality rates for these patients were 48.4% and 55.2%, respectively [2].

Despite early diagnosis and initiation of antibiotics, mortality remains high [3], probably owing to the existence of several other host-related factors that keep the septic reaction intense, leading to multiple organ dysfunction syndrome (MODS). Part of this intensity is attributed to the host–parasite interaction and to the deterioration of the host evoked by the immunological overreaction [4].

The need to restrain the hyperactivity of the host's immune system led to the generation of the idea of immunomodulation. The present review attempts to present how intravenously administered macrolides are candidates for immunointervention in sepsis based both on experimental and clinical evidence. Special emphasis is given to the application of clarithromycin as an immunomodulatory treatment.

2. **Evolution of immunomodulatory therapies in sepsis: a brief overview**

The idea of immunointervention in sepsis has evolved over the last two decades and was based on a rather simplistic scheme of pathogenesis. According to that scheme, the septic reaction is initiated when bacterial products such as endotoxin (lipopolysaccharide (LPS)), lipoteichoic acid, zymosan or even bacterial DNA stimulate blood monocytes and tissue macrophages through binding to Toll-like receptors embedded in the monocyte cell membranes. Following stimulation of intracellular signalling pathways leading to activation of nuclear factor (NF)-κB, gene expression of pro- and anti-inflammatory cytokines takes place, among which tumour necrosis factor-alpha (TNFα), interleukin (IL)-1β, IL-6, IL-8 and IL-10 are the most widely known [4]. Overproduction of these cytokines may lead to MODS, disseminated intravascular coagulation and hyperglycaemia.

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Based on the above scheme, it was thought that improved patient outcome could result by inhibiting one or more components of the inflammatory cascade. As a consequence, blocking or modulation of the immune response was attempted on the following targets: (a) LPS, with anti-endotoxin antibodies; (b) TNFα, with antibodies and soluble receptors; (c) coagulation pathways by the administration of drotrecogin alpha; (d) hyperglycaemia by intensive insulin therapy; and (e) restoration of adrenal insufficiency by administration of low doses of hydrocortisone [5].

Eleven randomised trials have already been performed with the administration of anti-endotoxin antibodies [5,6], anti-TNF antibodies [7–9] and TNFα soluble receptors (etanercept/lenerecept) [10,11]. More than 3000 patients have been enrolled in these studies. Failure to denote a clinical benefit of the administered therapy over placebo with regard to the 28-day survival was a common denominator for these studies.

Enthusiasm for the clinical benefit from an immune-targeted therapy was again created after publication of the results of the PROWESS study [12]. A total of 1690 patients with signs of one or more organ failures arising within <12 h were randomly allocated to either placebo or a 4-day continuous infusion of drotrecogin alpha (human recombinant activated protein C). The 28-day survival was either placebo or a 4-day continuous infusion of drotrecogin alpha; (d) hyperglycaemia by intensive insulin therapy; and (e) restoration of adrenal insufficiency by administration of low doses of hydrocortisone [5].

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Insulin therapy was accepted with much enthusiasm when it was found to reduce significantly in-hospital and ICU mortality in patients with severe sepsis [5]. However, when trying to repeat these results in a multicentre study of 537 patients, the trial was prematurely stopped for safety reasons owing to the advent of severe and life-threatening hypoglycaemia [14].

Among the above-described clinical attempts at immunomodulation, administration of low doses of hydrocortisone in patients with septic shock and relative adrenal insufficiency was beneficial in achieving earlier withdrawal of vasopressors and in reducing relative risk for death [15]. However, a recent randomised trial of 499 patients failed to demonstrate a reduction in risk of death by septic shock, although earlier resolution of shock was seen [16].

3. Why macrolides may be candidates for immunointervention in sepsis

Many hypotheses have been developed to provide an explanation for the unfavourable outcome of the abovementioned trials of immunomodulatory therapies in sepsis. Three probable explanations may be given [5]. (a) Evolution of the applied agents is based on animal models of pre treatment. In these models, the immunomodulator is administered in the host prior to challenge by the offending pathogen or the bacterial product. Although survival is prolonged and the inflammatory reaction is attenuated leading to a promising result from a pharmacological point of view, the experimental scenario is long away from clinical practice where immunointervention has to be administered in a patient with already settled signs of sepsis and organ failure. (b) Clinical studies have enrolled patients with different types of infections as an underlying cause of sepsis, thus leading to considerable heterogeneity of the study populations. And (c) the efficacy of the co-administered antimicrobial therapy appears to be neglected in the final analysis of these trials.

Based on the above type of reasoning, we tried to select an effective agent for immunointervention and to evaluate whether it fulfils several criteria for application in clinical trials of sepsis. We believed that macrolides may belong to that type of agents, as proven by more than 20 years of their clinical application in chronic inflammatory lung disorders. A prototype for these disorders is diffuse panbronchiolitis (DPB). This is a disease mainly seen in people living in Japan and is genetically inherited. It is characterised by progressive obstruction of the airways, which are colonised by mucus isolates of Pseudomonas aeruginosa. Within the first 20 years of their lives patients develop cor pulmonale and die. The introduction of erythromycin in 1979, followed by clarithromycin in 1995, significantly prolonged survival and reduced mortality from this disease [17].

Benefit disclosed in patients with DPB led to the search for other applications of macrolides in chronic inflammatory lung disorders characterised by obstruction of the bronchi, such as chronic obstructive pulmonary disease, cystic fibrosis (CF) and bronchiectasis. Four randomised trials have already been published [18] where azithromycin was administered to children with CF, accompanied by reduction of infectious exacerbations and subsequent increase in forced expiratory volume in the first second (FEV1). The mode of action of macrolides may involve several different targets [19]: (a) stabilisation of tight junctions between bronchial epithelial cells; (b) reduction of mucus produced by colonisers such as P. aeruginosa through inhibition of quorum sensing; (c) promotion of phagocytosis by alveolar macrophages; and (d) attenuation of the inflammatory reaction of the airways through reduction of the release of pro-inflammatory cytokines by airway epithelial cells.

However, all the above situations are characterised by chronic inflammation and do not provide enough evidence about a beneficial effect of macrolides in an acute septic state. In vitro evidence for a future application is derived from cultures of human monocytes. Release of IL-8 after stimulation with cell lysates of Escherichia coli and P. aeruginosa is reduced upon addition of clarithromycin at concentrations ranging between 1 μg/mL and 10 μg/mL [20]. The effect of clarithromycin is mediated through inhibition of the transcription factors NF-kB and activator protein 1 (AP-1).

The probability of an immunomodulatory effect of macrolides in acute inflammatory conditions is further enhanced by retrospective analysis of populations treated for community-acquired pneumonia. Among 1518 patients in Spain, the final outcome of those who were treated with a combination of a β-lactam and a macrolide was compared with those treated with β-lactam monotherapy [21]. Overall mortality was 6.9% vs. 13.3%, respectively (P = 0.001). Similar differences were encountered even for patients with a Pneumonia Severity Index (PSI) of 4 or 5. They were also seen in a subanalysis comprising only patients infected by Streptococcus pneumoniae in order to exclude a direct bactericidal effect on atypical pathogens as the reason for the macrolide benefit on overall mortality. Among 409 patients with bacteraemia by S. pneumoniae, the hazard ratio for death was reduced by 2.5 times when a combination of a β-lactam and a macrolide was administered compared with β-lactam monotherapy [22]. This was further verified in a retrospective cohort of 2349 patients with bacteraemic pneumonia [23].

Based on the hypothesis that serum levels close to 10 μg/mL are needed for modulation of the release of pro-inflammatory cytokines by monocytes, it was decided to apply clarithromycin for experimental studies instead of azithromycin since the latter fails to produce high serum levels [19]. Experimental pyleonephritis and sepsis were induced by susceptible E. coli, multidrug-resistant P. aeruginosa and pandrug-resistant Klebsiella pneumoniae, which are
bacterial species not belonging to the antimicrobial spectrum of clarithromycin [24–29]. Therapy with intravenous clarithromycin was started either in parallel with bacterial challenge or upon presentation of signs of sepsis in an attempt to simulate clinical practice and to avoid problems of interpretation of results occurring with former experimental studies of pre treatment. Results revealed that survival of animals administered clarithromycin was significantly prolonged. Although clarithromycin did not affect bacterial growth in tissues, the tissue inflammatory reaction was attenuated as evidenced by the degree of infiltration by neutrophils and lymphocytes. This was accompanied by a reduction in serum concentrations of TNFα as well as serum oxidant status, whereas ex vivo release of TNFα by peripheral blood monocytes was significantly decreased. Moreover, maximum serum concentrations of clarithromycin estimated 30 min after the end of infusion were within 5–10 μg/mL. This is consistent with the working hypothesis that changes of serum pharmacokinetics are mandatory to achieve a potent immunomodulatory effect of clarithromycin in an acute septic state.

4. Clinical efficacy of clarithromycin in patients with sepsis and ventilator-associated pneumonia

Based on the favourable results of the immunomodulatory effect of intravenously administered clarithromycin in experimental models of sepsis caused by Gram-negative clinical isolates, a randomised clinical trial was designed (http://www.clinicaltrials.gov; NCT00297674). The trial was designed to provide as much homogeneity as possible with regard to the underlying cause of sepsis in the study population. A total of 200 patients with nosocomial sepsis due to ventilator-associated pneumonia (VAP) were enrolled. Patients were randomly assigned to receive either placebo or clarithromycin. The latter was administered at a dose of 1 g daily within 1 h of infusion for three consecutive days. This type of regimen was expected to provide serum levels within 5–10 μg/mL according to preliminary pharmacokinetic studies [30]. The type of co-administered antimicrobial therapy was selected by the attending physicians. Sepsis, severe sepsis and septic shock were defined according to international definitions [31].

Results of that trial have recently been published [32]. From the total patients, 100 were allocated to treatment with placebo and 100 to treatment with clarithromycin. Both groups were well matched for age, sex, underlying diseases, severity of septic syndrome and organ failure. They were also well matched for the degree of respiratory dysfunction as assessed by the partial oxygen pressure (pO2)/fraction of inspired oxygen (FiO2) ratio and for the administration of other immunomodulatory therapies. The latter specify the rate of patients in each arm of treatment treated with low doses of hydrocortisone and with continuous infusion of insulin to achieve glucose levels within 80–110 mg/dL.

Assessment of the bacterial causes of VAP in the study population was performed by quantitative cultures of tracheobronchial secretion (TBS) before the start of the investigational drug; this was also repeated on Days 5 and 10. Among placebo-treated and clarithromycin-treated patients, pathogens at counts >10^5 colony-forming units/mL of TBS were isolated in 68% and 66%, respectively (not significant), all of which were Gram-negative isolates. More specifically, the commonest causative species of VAP among placebo-treated patients were Acinetobacter baumannii (63.2%), P. aeruginosa (17.6%) and K. pneumoniae (8.8%). Respective isolation rates among clarithromycin-treated patients were 54.5%, 25.8% and 7.6%.

The next step in the analysis was to exclude the type of administered antimicrobial as a confounding factor in interpretation of results. This was based on the rate of isolates in each study group being susceptible to one or more of the administered agents, which was 62.7% and 75.4% among placebo-treated and clarithromycin-
treated patients, respectively ($P = 0.44$). Eradication of the offending pathogens was achieved on Day 5 in 37.3% and 42.6% of patients, respectively ($P = 0.31$) and on Day 10 in 41.4% and 46.2% of patients, respectively ($P = 0.82$).

The following outcomes were compared between both treatment groups: resolution of VAP; time to de-intubation from mechanical ventilation; and risk of death. Analysis for the first two endpoints was done among survivors of each group. Median time to resolution of VAP was 15.5 days in the placebo group compared with 10.0 days in the clarithromycin group ($P = 0.011$). In the same context, median time to de-intubation was 22.5 days and 16.0 days, respectively ($P = 0.047$). These findings of earlier time to resolution of VAP and earlier de-intubation achieved with the administration of clarithromycin are consistent with the changes in the Clinical Pulmonary Infection Score (CPIS). CPIS was estimated for all enrolled patients. The mean CPIS at baseline was 7.92 and 7.62 among placebo-treated and clarithromycin-treated patients ($P = 0.29$), respectively, which were 6.10 and 5.23 on Day 5 ($P = 0.016$) and 5.88 and 5.09 on Day 10 ($P = 0.032$), respectively.

Survival analysis was based on the identification of the most important driver to death in the total population, which was the coexistence of septic shock and MODS. The odds ratio for death due to these causes was 19.00 among placebo-treated patients; it was reduced to 3.78 among clarithromycin-treated patients ($P = 0.043$).

In total, 22 patients developed MODS after enrolment in the study while having sepsis without signs of organ failure on the day of enrolment. Mean time to progression to MODS was 3.38 days among those who were treated with placebo; it was extended to 5.78 days for those treated with clarithromycin ($P = 0.006$).

No risk for serious adverse events was found among patients treated with clarithromycin.

These findings render some probable future indications of intravenously administered clarithromycin in patients with sepsis and VAP. The proposed indications are: (a) VAP, owing to earlier resolution and earlier weaning from mechanical ventilation; and (b) septic shock and MODS, owing to the significant reduction in the risk of death.

Various hypotheses could be proposed regarding the probable mechanism of action of clarithromycin (Fig. 1). Based on existing knowledge already described above for the effect of macrolides in chronic inflammatory lung disorders and in experimental models of sepsis, these may involve either a modulation of the immune function of the host or an effect on bacterial quorum sensing. We believe that the latter mechanism of action is unlikely since quorum sensing is characteristic of P. aeruginosa, which was not the predominant pathogen in our study population. Prolongation of the time to progression to MODS achieved by clarithromycin is an indirect sign pointing towards an immunomodulatory effect.

5. Conclusions

Severe sepsis and septic shock are among the leading causes of death worldwide. Despite intense research in the field and the application of a variety of therapies targeted at modulating the immune response of the host, mortality remains highly unaltered. The favourable findings of the analysed double-blind, randomised clinical trial of intravenously administered clarithromycin in patients with VAP and sepsis render us optimistic for the future. However, further research is mandatory to define the exact indications for clarithromycin as an immunomodulatory treatment of sepsis.

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**References**


