

INTRAVENOUS CLARITHROMYCIN: A VALUABLE IMMUNOMODULATOR FOR SEVERE INFECTIONS

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Primljen/Received 09. 03. 2014. god.

Prihvaćen/Accepted 02. 04. 2014. god.

Abstract: There are many studies suggesting that intake of a macrolide in the treatment regimen is linked with improved outcomes for patients with community-acquired pneumonia. However this was never proved through one randomized clinical study (RCT). We reviewed all pre-clinical and clinical development from 2003 to 2014 for intravenous clarithromycin as adjunctive treatment of severe infections. This process ended with the conduct of two RCTs; the first in 200 patients with ventilator-associated pneumonia; and the second in 600 patients with proven or suspected Gram-negative infections of non-pulmonary origin. The two RCTs has similar findings; significant decrease of mortality of patients with septic shock and multiple organ dysfunctions; and shorter resolution of severe infections. The second RCT also showed significant savings for survivors. These data support the use of intravenous clarithromycin as adjunctive treatment of severe infections.

Key words: clarithromycin, cytokines, inflammation, sepsis

INTRODUCTION

Despite increasing antimicrobial resistance, intake of a macrolide is connected with improved outcomes of patients with community-acquired pneumonia (CAP) (1–5). Benefit is even pronounced among patients with bacteremia by macrolide-resistant *Streptococcus pneumonia* (5) leading people to consider that this comes from modulation of the immune response of the host by macrolides. However, the definite proof may come only from randomized clinical trials (RCTs). No RCT has ever been conducted to prove benefit of macrolides intake in the outcome of severe CAP. Despite the lack of such RCT, the updated Surviving Sepsis Campaign guidelines recommend the use of a macrolide for the management of CAP complica-

ted by bacteremia from *Streptococcus pneumoniae* (6), giving emphasis to the validity of this intervention.

Our group has launched a research program since 2003 to explore the modulation of the immune response of the host and the benefit that is shown for patients with severe infections when intravenous clarithromycin is added in the treatment regimen. The present review outlines the main findings of these research efforts.

ANIMAL STUDIES

The efficacy of intravenously administered clarithromycin was studied in a series of pre-clinical animal studies. Results of these studies are summarized in Table 1 (7–11). The main infection models studied were pyelonephritis, pleuritis and peritonitis after cecal ligation and puncture. For pyelonephritis and pleuritis bacterial challenge was done by multidrug- and pandrug-resistant isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* against which macrolide do not possess any antimicrobial activity. Results from these animal studies 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 when administered in rabbits when signs of pulmonary edema appear (10). In pyelonephritis by multidrug-resistant *P.aeruginosa* and pandrug-resistant *K.pneumoniae*, histology showed that clarithromycin attenuated: a) peribronchial inflammation in the lung; b) mononuclear infiltration and necrosis in the liver and kidney; and c) activation of B- and T-cell rich areas in spleen (10, 11). The common denominator of all these studies was that monocytes and lymphocytes appeared to be the main target of action of clarithromycin.

In one model of pleuritis induced by multidrug-resistant *P.aeruginosa*, specimens of pleural fluid of rab-

Table 1. Summary of pre-clinical studies assessing the efficacy of intravenous clarithromycin in acute infection models

Reference	Experimental model	Microorganism	Survival	Bacterial growth	Circulating mediators	Mononuclears	Histology
7	Pyelonephritis	MDR <i>Pseudomonas aeruginosa</i>	Prolongation when co-administrated with amikacin	No effect	↓TNF α , MDA	N/A	N/A
8	Pyelonephritis	Susceptible <i>Escherichia coli</i>	Prolongation as pretreatment and treatment	No effect	↓ TNF α , MDA	↓monocytic caspase-3	N/A
9	Pyelonephritis	Susceptible <i>Escherichia coli</i>	Prolongation when co-administered with amikacin at signs of sepsis	No effect	No effect	N/A	N/A
10	Pyelonephritis	MDR <i>Pseudomonas aeruginosa</i>	Prolongation when co-administrated with amikacin at signs of sepsis	No effect	No effect	↓ ex vivo production of TNF α by monocytes	↓lung-spleen inflammation
11	Pyelonephritis	PDR <i>Klebsiellapneumoniae</i>	N/A	No effect	↓TNF α , MDA	↓ ex vivo production of TNF α by monocytes	↓kidney-liver inflammation
12	Peritonitis by CLP		Prolongation when co-administrated with piperacillin/ tazobactam	No effect	N/A	↓ ex vivo production of TNF α , ↓ apoptosis lymphocytes and monocytes	N/A
13	Pleuritis	MDR <i>Pseudomonas aeruginosa</i>	N/A	↓ lung and pleural fluid	↓TNF α	↓ production of TNF α and IL-6	

Abbreviations: CLP: cecal ligation and puncture; IL: interleukin; MDA: malondialdehyde; MDR: multidrug-resistant; N/A: not assessed; PDR: pandrug-resistant, TNF α : tumor necrosis factor-alpha ;↓: decrease

bits were used to stimulate cytokine production from U937 monocytes. It was shown that those specimens coming from animals treated with a combination of clarithromycin and piperacillin/tazobactam produced much lower concentrations of tumour necrosis factor-alpha (TNF α) and of interleukin (IL)-6 compared with the amount of cytokines released from specimens coming from rabbits administered single piperacillin/tazobactam (13). This finding drives the hypothesis that part of the mode of action of clarithromycin is mediated through attenuated release of pathogen-associated molecular patterns by *P.aeruginosa*.

INTRAVENOUS CLARITHROMYCIN IN SEPSIS: CLINICAL EVIDENCE

The promising results of experimental studies led us to design and conduct two randomized clinical trials

to explore the immunomodulatory effect of clarithromycin in patients with sepsis. It is suggested 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. To overcome this, the first trial enrolled patients suffering from the same underlying infection, namely ventilator-associated pneumonia (VAP). Two hundred patients with sepsis and VAP were enrolled in a double-blind, randomized, multicenter trial from June 2004 until November 2005 (14). Clarithromycin (1 g) was administered intravenously once daily for 3 consecutive days in 100 patients; another 100 patients were treated with placebo. Main outcomes were resolution of VAP, duration of mechanical ventilation, and sepsis-related mortality within 28 days. There were no differences between groups in baseline characteristics. Microbiology was also assessed

and Gram-negative bacteria growing at counts greater than 10^5 cfu/ml of tracheobronchial secretions (TBS) were considered. Gram-negative bacteria 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 and *P.aeruginosa* in 12 and 17 patients. Initial empirical antimicrobial coverage was appropriate 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). TBS were sampled again at follow-up. Eradication of the pathogen was achieved in 25.4% and 33.8% of cases on day 5 ($p = 0.31$ between groups) and in 31.3% and 29.2% of cases 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 for death from septic shock and multiple organ failure was 19.00 in placebo-treated patients; it was reduced to 3.78 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 scores ($p = 0.011$). Mean clinical pulmonary infection score for the placebo and the clarithromycin group on study enrolment were 7.92 and 7.62, respectively ($p = 0.29$). It was 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 ($p = 0.049$).

To confirm these findings in another patient population, a second RCT was conducted in 600 patients with proved or suspected Gram-negative infections i.e. primary Gram-negative bacteremia, acute pyelonephritis and acute intrabdominal infections aggravated by systemic inflammatory response syndrome (15). Clarithromycin blind treatment was assigned to 302 patients; 298 patients were treated with placebo. In this RCT, period of treatment was prolonged from three to four days. The primary endpoint was mortality by severe sepsis/shock and MODS; resolution of infection and hospitalization costs were the secondary endpoints. Demographics and sepsis severity were similar between groups. The most common pathogens were Gram-negative bacteria namely *E.coli* (36.2% of bloodstream pathogens in the placebo arm and 43.8% of bloodstream pathogens in the clarithromycin arm), *K.pneumoniae* (21.2% and 11.2% respectively), *P.aeruginosa* (10.0% and 6.7% respectively) and *A.ba-*

umannii (11.2% and 6.7% respectively). The time from sepsis onset to start of antimicrobials and appropriateness of antimicrobials did not differ between groups.

Overall 28-day mortality was similar between groups being 17.1% in the placebo group and 18.5% in the clarithromycin group. Mortality of placebo-treated patients with septic shock and MODS was 73.1% compared to 53.6% of clarithromycin-treated patients ($p = 0.020$). The median time until resolution of infection was 10 days among patients with severe sepsis/shock treated with placebo and 6 days among patients with severe sepsis/shock treated with clarithromycin ($p = 0.037$). The median cost of hospitalization was € 3383.5 for survivors of the placebo arm and € 2269.3 for survivors of the clarithromycin arm ($p = 0.044$). Serious adverse events were observed in 1.3% and 0.7% of placebo- and clarithromycin-treated patients, respectively ($p = 0.502$).

The findings of the second trial fully confirm results of the first trial. Intervention with clarithromycin decreased mortality by septic shock and MODS and shortened the time until resolution of Gram-negative infections worsened by severe sepsis or septic shock. As expected, earlier resolution of severe Gram-negative infections was accompanied by significant savings for survivors. More precisely, median savings for every survivor were € 1114.3. The studied intervention costs € 70 per patient.

WHAT IS THE MECHANISM OF ACTION OF MACROLIDES?

It is traditionally conceived that macrolides attenuate the inflammatory response of the host. Most of conducted studies in animals including those used for the pre-clinical development of intravenous clarithromycin in sepsis support this concept. Our animal studies indicate that treatment with intravenous clarithromycin leads to decreased circulating levels of pro-inflammatory mediators and to attenuation of the mononuclear tissue infiltrates (Table 1). To the same end, studies of infected mice show that pre-treatment with azithromycin is accompanied by decreased concentrations of pro-inflammatory cytokines in the systemic circulation and in the lung (16, 17, 18). It is well-known that macrolides attenuate pro-inflammatory phenomena in the lung where they accumulate. These properties may well explain the benefit shown in patients with VAP of the first RCT but not in patients of the second RCT who were not sufferers from any lung infection.

To explore the effect of intravenous clarithromycin in the inflammatory cascade in the first trial of patients with VAP (19), circulating cytokines were

measured before allocation to blind treatment and for six consecutive days. On the same time intervals, circulating monocytes were isolated and stimulated for cytokine production. Expression of the co-stimulatory molecule CD86 involved in antigen presentation was also measured on the cell membranes of monocytes. Contrary to what was expected, the ratio of circulating IL-10 to TNF α was decreased on day 4 in the clarithromycin arm compared to the placebo arm. On this same day, expression of CD86 on monocytes was increased as was also their ability for the production of IL-6 after stimulation with bacterial ligands. These findings were much pronounced among patients with septic shock and MODS for whom most of survival benefit was shown. This tight time connection between treatment and effect may indicate that clarithromycin is a modulator of the biological function of monocytes so as to corroborate animal studies. However these findings also suggest that in the clinical field intravenous clarithromycin modulated the function of monocytes towards reversal of sepsis-induced immunoparalysis of patients. The ratio of pro-inflammatory to anti-inflammatory cytokines was restored, antigen-presentation was more efficient and monocytes became fully functional for the production of pro-inflammatory cytokines.

It is now widely recognized that when severe sepsis and MODS develop, the host has entered into a state of immunoparalysis (20). The key components of sepsis-induced immunoparalysis are failure of monocytes for adequate cytokine production after stimulation and defective antigen presentation. These defects were

fully restored in patients with VAP treated with clarithromycin particularly those with septic shock and MODS.

CONCLUSIONS

Despite the existence of many retrospective analysis showing improved outcome of severe CAP when a macrolide is added to the treatment regimen of CAP, no RCT is available to definitively prove this benefit. Despite the lack of such RCT, the updated Surviving Sepsis Campaign guidelines recommend the use of a macrolide for the management of CAP complicated by bacteremia from *Streptococcus pneumoniae*. The only available RCTs in critically ill patients compare the efficacy of intravenous clarithromycin over placebo when added to the standard of care of ventilator-associated pneumonia and of Gram-negative infections of non-pulmonary origin. Common findings of both RCTs are decrease of mortality and earlier resolution of infection of the most severe patients. These findings definitively support a novel therapeutic indication of intravenous clarithromycin for the severely ill patients.

Abbreviations

CAP — community-acquired pneumonia

IL — interleukin

MODS — multiple organ dysfunction syndrome

RCT — randomized clinical trial

TNF α — tumour necrosis factor-alpha

VAP — ventilator-associated pneumonia

Sažetak

INTRAVENSKA PRIMENA CLARITHROMYCIN-A: DRAGOCEN IMUNOMODULATOR KOD TEŠKIH INFEKCIJA

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Postoji mnogo studija koje sugerišu da je primena makrolida u lečenju upale pluća povezana sa boljim ishodom. Ipak, ovo nije nikada dokazano kroz jednu randomiziranu kliničku studiju. Pregledali smo sve prekliničke i kliničke rezultate od 2003. do 2014. godine, vezane za intravensku primenu klaritromicina kao dodatnog lečenja teških infekcija. Ovaj proces se završio sprovođenjem dve randomizirane kliničke studije; prva je uključila 200 pacijenata na mehaničkoj ventilaciji, sa upalom pluća; druga je uključila 600 pacijenata sa

dokazanom ili sumnjivom Gram-negativnom infekcijom koja nema plucno poreklo. Dve randomizirane kliničke studije su imale slične rezultate; značajno povećanje mortaliteta pacijenata sa septičkim šokom i multiorganskom disfunkcijom; i kraće lečenje teških infekcija. Druga randomizirana klinička studija je pokazala značajnu uštedu za preživjele. Ovi podaci podržavaju intravensku primenu klaritromicina kao dodatno lečenje teških infekcija.

Ključne reci: klaritromicin, citokini, upala, sepsa.

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