A new mechanism of action, via formation of Neutrophil Extracellular Traps, for the widely used and established macrolide antibiotic Clarithromycin

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Introduction

Macrolides have been demonstrated as potential immunomodulatory agents, due to their ability to induce the activity of various immune cells and their regulatory role in chemokine and cytokine production. Although it was proposed that some macrolides could induce degranulation and bacterial killing in neutrophils, knowledge regarding their role in these cells has been very limited. In patients with clarithromycin (macrolide antibiotic) resistant bacterial infections, combined treatment with clarithromycin was positively correlated with the time of resolution of the infection. Neutrophils are the most abundant circulating inflammatory cells and the first line of defense against pathogens. They employ three major strategies to fight against microbes: phagocytosis, degranulation, and the release of neutrophil extracellular traps (NETs). The discovery of the NET formation mechanism has redefined our perception of the role and functions of neutrophils. NETs are composed of chromatin which is decorated with neutrophilic proteins, such as myeloperoxidase (MPO), neutrophil elastase (NE) and LL-37 (antimicrobial peptide). NEs not only exert their antimicrobial effect through pathogen immobilization by entrapment but also have a direct microbicidal effect that depends on the formation, movement, and migration of NETs.

On the other hand, it has been also reported that some microorganisms, such as Acinetobacter baumannii, escape NETs or possess inhibitory strategies against NET formation mechanism. For this reason, we hypothesized that clarithromycin could induce NETosis in the presence of Acinetobacter baumannii. This study was designed to investigate the role of clarithromycin in the generation of NETs and whether NETs are affected by clarithromycin.

Objective of the study

• Investigation of the role of clarithromycin in the generation of NETs.
• Investigation of how clarithromycin derived NETs could affect pathogens that are not able to trigger the mechanism of NETosis and are resistant to clarithromycin, such as Acinetobacter baumannii.

Methods

Blood sample collection

Healthy individual

Helicobacter pylori positive gastritis patients

Isolation of neutrophils

In vitro stimulation with clarithromycin

Ex vivo neutrophil culture

Production of NET structures & NET proteins

Results

Figure 2. In vitro stimulation of neutrophils from healthy individuals with clinically relevant concentrations of clarithromycin induced NET formation.

Figure 3. Neutrophils during clarithromycin therapy demonstrated increased ex vivo NET formation compared to neutrophils before clarithromycin initiation and neutrophils from healthy individuals.

Figure 4. NETs released by neutrophils from control subjects treated with clarithromycin were decorated with LL-37. LL-37 was absent from NETs induced by monosodium urate (MSU) (general NET inducer). Neutrophils derived from patients treated with clarithromycin also demonstrated presence of LL-37 on NETs.

Figure 5. Both in vitro and ex vivo clarithromycin-induced NETs significantly reduced bacterial growth of Acinetobacter baumannii compared to control cultures. LL-37 neutralization with anti-LL-37 antibody abolished the antimicrobial action of clarithromycin-induced NETs. MSU induced NETs did not have the same antimicrobial activity.

Conclusions

• Clarithromycin induces NET generation in vitro and in vivo
• LL-37 is present on NETs induced by clarithromycin both in vitro and in vivo
• Both in vitro and in vivo clarithromycin-induced NET inhibit Acinetobacter baumannii in a LL-37 dependent manner.

References