PERSPECTIVE IN MEDICINAL CHEMISTRY

Azithromycin Use in COVID-19 Patients: Implications on the Antimicrobial Resistance

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1. AZITHROMYCIN AND COVID-19

Azithromycin (AZ) is a broad-spectrum second-generation macrolide with an extensive tissue distribution, primarily used for the treatment of respiratory, enteric and genitourinary bacterial infections, such as community-acquired pneumonia and chlamydia [1-3]. As the world faces the coronavirus disease 2019 (COVID-19) pandemic, researchers are urgently attempting to identify drugs to treat the disease using different approaches, including the repurposal of approved compounds and evaluation of their activity against the ethological agent, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 4, 5]. In this context, AZ presents antiviral and immunomodulatory properties that seem promising in the treatment of COVID-19, with some studies showing *in vitro* activity of this drug against the SARS-CoV-2 [6, 7]. Diverse mechanisms have been proposed for the antiviral properties of AZ, including interaction with receptors (*e.g.,* angiotensin converting enzyme 2 – ACE2 [6], and CD147 [5]), inhibition of endocytosis and uncoating of enveloped viruses [1, 2, 7, 9-11], mucociliary clearance improvement [12-14] and immunomodulatory activity [2, 7, 15-19]. AZ may increase type I interferon expression, crucial for restricting viral replication and spread [2, 7, 20-22], and it may also improve the host viral recognition system by upregulating genes such as the ones encoding MDA5 and RIG-I [7, 20]. Moreover, AZ administration seems to downregulate pro-inflammatory cytokines (such as interleukin (IL)-6, IL-8, IL-1β and tumor necrosis factor alpha) [2, 7, 15-19], which may attenuate the onset of cytokine release syndrome related to COVID-19 [7]. Other anti-inflammatory mechanisms include the decrease of active neutrophil subpopulations [17], the suppression of $CD4^+$ T-cell activation [23], and the repolarization of alveolar macrophages towards their activated anti-inflammatory M2 phenotype [7, 24]. Therefore, AZ anti-inflammatory and immunomodulatory properties, added to its ability to prevent lung fibrosis and to maintain epithelial integrity, may play a role in the control of hyperinflammation in COVID-19 [6, 7].

Despite AZ being a promising therapy, studies are still needed to better evaluate its use in COVID-19. The lack of adjusted comparison and control groups in most observational studies is relevant given some confounding factors, such as the use of other therapies (*e.g.,* antivirals, corticosteroids, anticoagulation therapies, and chloroquine\hydroxychloroquine) [6, 25]. In this context, clinical trials, particularly the randomized double-blinded controlled ones, are crucial to evaluate the safety and efficacy of treatment or prevention approaches [5]. Therefore, the future outcomes of such studies are fundamental to establish the role of AZ in the treatment of COVID-19, including the optimal stage of use, the posology and the effects of its combination with other drugs [26]. According to the information available on *ClinicalTrials.gov*, to date, 43 clinical trials are recruiting patients to evaluate AZ in COVID-19 therapeutics, considering diverse scenarios. The RECOVERY trial, for example, is investigating the use of 500 mg of AZ intravenously or by mouth (or nasogastric tube) once daily during 10 days in COVID-19 patients [28]. Moreover, to date, 11 clinical trials that considered AZ in their scope are completed, of which two are submitted, but not yet posted results, and one (NCT04321278) has published results [29]. This randomized open-label clinical trial pointed out that adding AZ to standard care treatment (which included hydroxychloroquine) did not result in clinical improvement or mortality reduction in patients with severe COVID-19 [29].

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2. MACROLIDE/AZ RESISTANCE: INCIDENCE, GENES AND RESISTANCE MECHANISMS

Macrolide resistance rates have substantially increased in many countries, since the therapeutic introduction of long-acting macrolides (particularly AZ) for community respiratory tract infections in the 1990s [30]. This resistance has been observed in *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus, Haemophilus* spp., *Mycoplasma pneumoniae, Neisseria gonorrhoeae*, among other microorganisms [30-42]. Despite the greatest concern regarding the high AZ resistance rates in *N. gonorrhoeae* in the context of sexually transmitted diseases [34-36], it is also important to consider the resistance rates of bacterial species associated with respiratory tract infections (RTIs). Macrolide resistance rates in those species are heterogeneous around the world, with most countries reporting resistance in at least 10% of *S. pneumoniae* clinical isolates [30]. China, for example, presents high macrolide resistance rates for *S. pneumoniae*: about 70% nationwide and over 90% in some regions [30, 39, 40]. A retrospective cohort study showed that AZ resistance was the most common antimicrobial resistance (51.4%) among 358 hospitalized adults in Missouri (USA), most of them related to RTIs [37]. Another retrospective study reported a significant increase in AZ resistance in *S. pyogenes* strains recovered from children with upper RTIs in Taiwan from 19.3% to 61.0% from 2000 to 2010 decade [33]. In addition, a cross-sectional study of 105 cases of pneumonia associated with mechanical ventilation showed that 73.2% (30/41) isolates of *Acinetobacter* spp., 66.7% (16/24) of *Klebsiella* spp., 72.2% (13/18) of *Pseudomonas* spp., 44.4% (4/9) of *Escherichia coli* and 50% (2/4) of *Proteus* spp. [43] were resistant to AZ. The same was reported for 40% of clinical isolates of *Klebsiella* spp. from 480 hospitalized patients in Iran [44]. These data are relevant considering the use of mechanical ventilation in COVID-19 patients with low oxygen saturation and the associated risk of nosocomial infections by these species.

Macrolide resistance is related to several mechanisms, including a macrolide efflux pump system (coded by *mef* genes) [45, 46] and ribosomal target modifications mediated by rRNA erm methylases (coded by *erm* genes, such as *erm*(A), *erm*(B), *erm*(C), *erm*(E) and *erm*(F)) [31-33, 47-52], conferring resistance in both Gram-positive and Gram-negative bacteria [49-51]. Some of these genes may persist on mobile genetic elements, facilitating their spread among different strains and species [53]. In addition, macrolide, lincosamide and streptogramin (MLSB) phenotype (related to *erm* genes) is usually associated with resistance to other antimicrobial classes, such as tetracyclines and chloramphenicol, since their resistance genetic determinants may be co-localized in the same mobile genetic element that is *erm*(*B*) [30, 54].

3. AZ AND ANTIMICROBIAL RESISTANCE (AMR)

Several studies reported a strong association between macrolide resistance and previous AZ usage [30, 55-63]. Some of them demonstrated that exposure to AZ increased patient's likelihood of harboring resistant strains for several weeks [61, 62], which may also be related to substantial re-infection rates [62]. In Canadian provinces, where AZ was the most commonly prescribed macrolide, it was observed a higher prevalence of macrolide resistant strains [58, 59]. Moreover, AZ long-term treatment in patients with chronic lung diseases increased the risk of bacterial resistance 2.7-fold compared to placebo treatment, supporting the idea that AZ long-term treatment may contribute to the development of bacterial resistance [63].

This association between AZ use and macrolide resistance may be explained by a pharmacodynamic parameter known as the mutant prevention concentration (MPC). MPC is essentially the lowest concentration of antimicrobial drug needed to inhibit the growth of the least susceptible bacterial cell in a bacterial population [64-73]. Therefore, mutant subpopulations are unlikely to be enriched if antimicrobial concentrations are kept above the MPC [74, 75]. In practical terms, the MPC is a measure of the minimum inhibitory concentration (MIC) that uses a more concentrated inoculum (final concentration ~10^9 colony-forming units – CFUs) in order to enhance the detection of resistant subpopulations, more accurately reflecting the dynamics of high-density bacterial populations [3, 65-67]. The frequency at which mutations occur is on the order of 1×10^{-7}–1×10^{-9}, and traditional susceptibility tests use an inoculum size of 10^5 CFUs. Therefore, an isolate considered susceptible to the MIC may contain an undetected subpopulation of resistant cells [3, 65, 66]. Based on the MIC and MPC values of a bacterial population exposed to an antimicrobial, the mutant selection window (MSW) may be established. MSW is the concentration range that inhibits the growth of susceptible cells, while selectively enriches non-susceptible mutants, and it is delimited by the MIC and the MPC [66-68, 70] (Fig. **1**). When antimicrobial concentrations are inside the MSW, the emergence of resistance is promoted. Consequently, reducing the interval of time that the antimicrobial concentrations remain in the MSW may decrease the probability of resistance emergence during therapy [64].

Based on these concepts, some studies used pharmacokinetics (PK) and pharmacodynamics (PD) parameters to explore possible considerations related to macrolide resistance [64-66, 76]. According to them, AZ seems to be intrinsically most likely to selectively enrich resistant mutant subpopulations, since this drug presents low values of area under curve over 24 h (AUC_{24})/MPC, C_{max}/MPC, and T_{MPC} compared to other antimicrobials (Table **1**) [65, 66, 76]. Compounds that are less likely to selectively enrich resistant mutants present higher AUC_{24}/MPC values [66]. AZ also persists inside the MSW for a long time (24 h) [66], and presents a long half-life (≈68 h), which may lead to prolonged sub-inhibitory serum and tissue concentrations [3, 61, 62]. Taken together, these settings may favor the selection of resistant mutant subpopulations, contributing to the emergence of macrolide resistance (Fig. **2**) [3, 65, 66, 76].

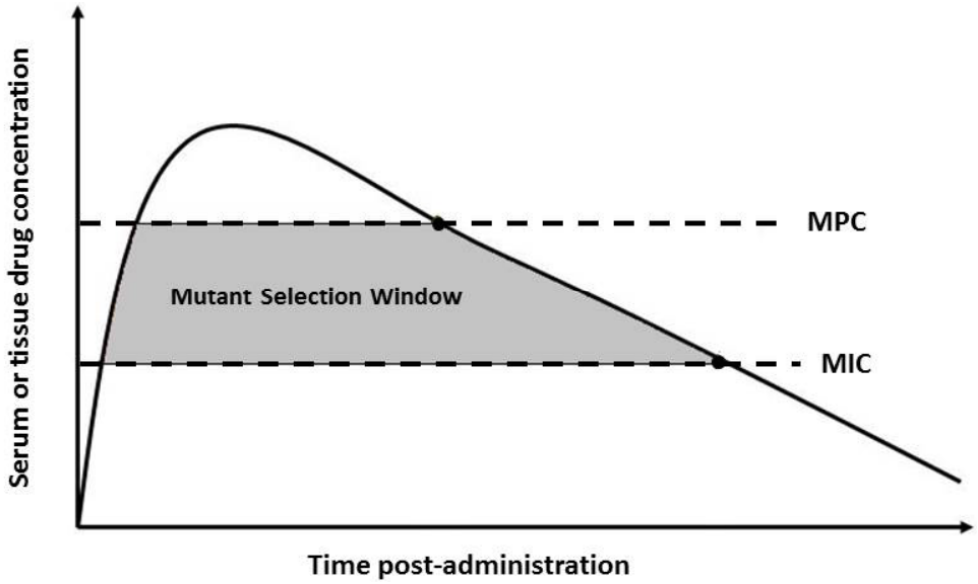

Fig. (1). The mutant selection window (MSW) is delimited by the minimum inhibitory concentration (MIC) and the mutant prevention concentration (MPC). This concentration range inhibits the growth of susceptible bacterial cells, while selectively enriches non-susceptible mutants [66-68, 70]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. Studies that evaluated MPC related parameters to azithromycin (compared to other antimicrobials).

References	Strains	Outcomes	
Metzler *et al.*, 2013	Clinical isolates of *Streptococcus pneumoniae* (n= 191)	**MPC_{90}** • Azithromycin = 4 • Clarithromycin = 0.5 • Erythromycin = 2 **AUC_{24} / MPC_{90}** • Azithromycin = 0.85 • Clarithromycin = 96.2 • Erythromycin base = 4 • Erythromycin estolate = 10.2 **Cmax / MPC_{90}** • Azithromycin = 0.1 • Clarithromycin = 7.5 • Erythromycin base = 0.45 • Erythromycin estolate = 6.2	**T>MPC_{90}** • Azithromycin = 0 • Clarithromycin = 24 • Erythromycin base = ~ 1 • Erythromycin estolate = ~ 5 **T_{MSW}** • Azithromycin =24 • Clarithromycin = 0 • Erythromycin base = 13 • Erythromycin estolate = 13
Blondeau *et al.*, 2015	Clinical isolates of *S. pneumoniae* (n=2) *S. pneumoniae* ATCC49616	**MPC** • Azithromycin = 0.5 – 2 • Clarithromycin = 0.25 – 0.5 • Erythromycin = 0.25 – 0.5 • Gemifloxacin = 0.125 – 0.25 • Telithromycin = 0.016 – 0.031	
Allen & Harris, 2017	*Shigella flexneri* m-12022 (isogenic **gyrA** mutant) *Shigella flexneri* ATCC12022	**AUC/MPC (m-12022 / ATCC 12022)** • Azithromycin = <0.1 • Ciprofloxacin = 10 / 77 • Levofloxacin = 16 / 66 • Moxifloxacin = 14 / 58	**%T >MPC (m-12022 / ATCC 12022)** • Azithromycin = 0 / 0 • Ceftriaxone = 0 / 31 • Ciprofloxacin = 2 / 100 • Levofloxacin = 28 / 87 • Moxifloxacin = 22 / 100

(Table 1) contd…..

References	Strains	Outcomes	
		AUC_{24} / MPC_{90}	**%T>MPC_{90}**
Berghaus *et al.*, 2013	Virulent strains of *Rhodococcus equi* (n=4)	• Rifampin = 0.3 • Erythromycin = 3.1 • Clarithromycin = 6.8 • Azithromycin = 0.3 • Amikacin = 0.6 • Gentamicin =13 • Enrofloxacin = 1.4 • Vancomycin = 54 • Imipenem = 0.5 • Doxycycline = 2.8	• Rifampin = 0 • Erythromycin = 0 • Clarithromycin = 0 • Azithromycin = 0 • Amikacin = 0 • Gentamicin =17 • Enrofloxacin = 0 • Vancomycin = 44 • Imipenem = 0 • Doxycycline = 0

AUC_{24}: area under curve over a 24 hours period; Cmax: serum maximum concentration; MIC: minimal inhibitory concentration; MPC: mutant prevention concentration; T_{MSW}: time inside the mutant selection window (h); T > MPC: interval of time that plasma concentration exceed the MPC; %T > MPC: the percentage of each dosage interval that plasma concentration exceed the MPC. Concentrations are in mg/L.

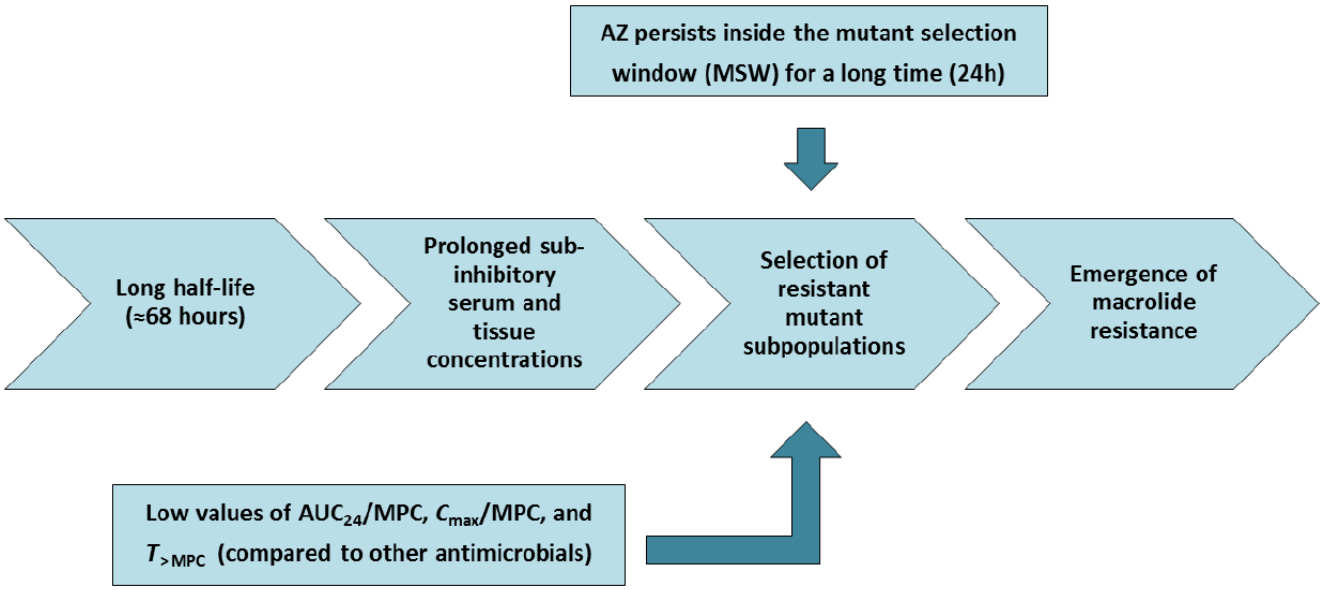

Fig. (2). Pharmacokinetics (PK) and pharmacodynamics (PD) settings of azithromycin (AZ) that seems to favor the selection of resistant mutant subpopulations, contributing to the emergence of macrolide resistance [3, 65, 66, 76]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

CONCLUSION

Therefore, the broad use of AZ in COVID-19, although under evaluation, may seriously impact on the increase of antimicrobial resistance. If AZ does not present a significant role in the therapeutics of COVID-19, avoiding its use would reduce unnecessary antibiotic consumption [77], corroborating with the rational use of antimicrobials proposed by the global plan to combat AMR.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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[27] completed Studies | covid19 | azithromycin - List Results https://clinicaltrials.gov/ct2/results?cond=Covid19&intr=Azithromycin&Search=Apply&recrs=e&age_v=&gndr=&type=&rslt=

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