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

Gabriela Seabra1,2, Roberta Ferreira Ventura Mendes1,2, Luiz Felipe Vieira dos Santos Amorim1, Ingrid Vianez Peregrino1,2, Marta Helena Branquinha3, André Luis Souza dos Santos3,4,* and Ana Paula Ferreira Nunes1,2,*

1Laboratório de Resistência Bacteriana (RESBAC), Departamento de Microbiologia, Centro de Ciências da Saúde (CCS), Universidade Federal do Espírito Santo (UFES), Vitória, Brazil; 2Programa de Pós-Graduação em Doenças Infecciosas, Universidade Federal do Espírito Santo (UFES), Vitória, Brazil; 3Laboratório de Estudos Avançados de Microrganismos Emergentes e Resistentes (LEAMER), Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; 4Programa de Pós-Graduação em Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

1AZITHROMYCIN 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 etiological 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, 8-11], 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 [27, 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].

*Address correspondence to these authors at the Laboratório de Resistência Bacteriana (RESBAC), Departamento de Microbiologia, Centro de Ciências da Saúde (CCS), Universidade Federal do Espírito Santo (UFES), Vitória, Brazil; E-mail: anastron@gmail.com (A.P.N.) and Laboratório de Estudos Avançados de Microrganismos Emergentes e Resistentes (LEAMER), Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; E-mail: andre@micro.ufrj.br (A.L.S.S.)
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 *ermA*, *ermB*, *ermC*, *ermE* and *ermF*) [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 *ermB* [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 ~10⁹ 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 10² 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 (AUC₉₆/MPC, Cmax/MPC, and T₉₀₆ compared to other antimicrobials (Table 1) [65, 66, 76]. Compounds that are less likely to selectively enrich resistant mutants present higher AUC₉₆/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).

<table>
<thead>
<tr>
<th>References</th>
<th>Strains</th>
<th>MPC$_{90}$</th>
<th>T &gt; MPC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metzler et al., 2013 [66]</td>
<td>Clinical isolates of <em>Streptococcus pneumoniae</em> (n= 191)</td>
<td>Azithromycin = 4, Clarithromycin = 0.5, Erythromycin = 2</td>
<td>Azithromycin = 0, Clarithromycin = 24, Erythromycin base = -1, Erythromycin estolate = -5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AUC$<em>{24}$/MPC$</em>{90}$: Azithromycin = 0.85, Clarithromycin = 96.2, Erythromycin base = 4, Erythromycin estolate = 10.2</td>
<td>T$_{MSW}$: Azithromycin = 24, Clarithromycin = 0, Erythromycin base = 13, Erythromycin estolate = 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cmax/MPC$_{90}$: Azithromycin = 0.1, Clarithromycin = 7.5, Erythromycin base = 0.45, Erythromycin estolate = 6.2</td>
<td></td>
</tr>
<tr>
<td>Blondeau et al., 2015 [65]</td>
<td>Clinical isolates of <em>S. pneumoniae</em> (n=2) <em>S. pneumoniae</em> ATCC49616</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Allen &amp; Harris, 2017 [76]</td>
<td><em>Shigella flexneri</em> m-12022 (isogenic gyrA mutant) <em>Shigella flexneri</em> ATCC12022</td>
<td>AUC$<em>{24}$/MPC$</em>{90}$: Azithromycin = &lt;0.1, Ciprofloxacin = 10 / 77, Levofloxacin = 16 / 66, Moxifloxacin = 14 / 58</td>
<td>%T &gt; MPC$_{90}$: Azithromycin = 0 / 0, Ceftriaxone = 0 / 31, Ciprofloxacin = 2 / 100, Levofloxacin = 28 / 87, Moxifloxacin = 22 / 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%T&gt;MPC$_{90}$: Rifampin = 0, Erythromycin = 2.8, Clarithromycin = 0.3, Amikacin = 0.6, Gentamicin =13, Enrofloxacin = 1.4, Vancomycin = 54, Imipenem = 0.5, Doxycycline = 2.8</td>
<td></td>
</tr>
<tr>
<td>Berghaus et al., 2013 [64]</td>
<td>Virulent strains of <em>Rhodococcus equi</em> (n=4)</td>
<td>AUC$<em>{24}$/MPC$</em>{90}$: 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</td>
<td>%T&gt;MPC$_{90}$: Rifampin = 0, Erythromycin = 0, Clarithromycin = 0, Azithromycin = 0, Amikacin = 0, Gentamicin =17, Enrofloxacin = 0, Vancomycin = 44, Imipenem = 0, Doxycycline = 0</td>
</tr>
</tbody>
</table>

Abbreviations: 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.

FUNDING

The present work was supported by grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação de Amparo à Pesquisa do Espírito Santo (FAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Financial code - 001), Brazil.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

Azithromycin Use in COVID-19 Patients

Current Topics in Medicinal Chemistry, 2021, Vol. 21, No. 8 681


Current Topics in Medicinal Chemistry, 2021, Vol. 21, No. 8


Azithromycin Use in COVID-19 Patients

Current Topics in Medicinal Chemistry, 2021, Vol. 21, No. 8 683

http://dx.doi.org/10.1186/s13756-020-00783-w PMID: 32723393


