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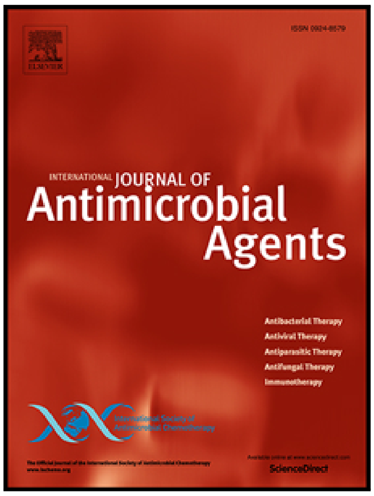

Macrolides and viral infections: focus on azithromycin in COVID-19 pathology

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Highlights
- There is the necessity to quickly find therapeutic options to treat novel SARS-CoV2
- Azithromycin has demonstrated to have antiviral and immunomodulatory effects, which could be effective in the hyper-inflammatory syndrome caused by SARS-CoV2
- Azithromycin has also shown clinical efficacy in respiratory distress syndrome and in viral infections
- Preliminary results regarding the efficacy of the combination of azithromycin and hydroxychloroquine in COVID-19 are conflicting
- There are some concerns regarding the association of azithromycin and hydroxychloroquine because of Qt prolongation
- Further studies have to be performed to investigate safety and efficacy of azithromycin and the combination with hydroxychloroquine in COVID-19

Macrolides and viral infections: focus on azithromycin in COVID-19 pathology

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Abstract

The emergence of the new disease COVID-19, is posing the challenge of seeking effective therapies. Since the most severe clinical manifestation of COVID-19 appeared to be a severe acute respiratory syndrome, azithromycin has been proposed as a potential treatment.

Azithromycin is known to have immunomodulating and antiviral properties. In vitro studies have demonstrated the capacity of azithromycin to reduce production of pro-inflammatory cytokines such as IL-8, IL-6, TNF alpha, reduce oxidative stress and modulate T-helper functions. At the same time there are multiple clinical evidences of the role of azithromycin in acute respiratory distress syndrome and against MERS. Some preliminary evidences have demonstrated controversial results regarding efficacy of azithromycin in combination with hydroxychloroquine in COVID-19. Firstly, a

French trial demonstrated 100% of virological negativization of six patients treated with azithromycin plus hydroxychloroquine vs 57.1% of patients treated with only hydroxychloroquine and 12.5% of the control group ($p<0.05$). On the other hand, another case series revealed no efficacy at all on eleven patients treated with same combination and doses.

Furthermore, there are some concerns regarding the association of azithromycin and hydroxychloroquine because of the potential Qt prolongation. In fact, both drugs have this as potential side effect and evidences regarding the safety use of this combination are controversial.

Despite the necessity to quickly find solutions for COVID-19, extreme caution must be used in evaluating the risk-benefit balance. However, based on preclinical and clinical evidences and some preliminary results in COVID-19, azithromycin could have a potential in the fight against this new disease.

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1. Introduction

Macrolides are bacteriostatic antibiotics widely used in clinical practice against many Gram-positive and atypical bacterial species that are commonly associated with respiratory tract infections. In addition to their antibacterial effects, macrolides have been shown to have immunomodulatory and anti-inflammatory effects [1-3]. The severity and mortality ~~caused~~ of respiratory viral infections including COVID-19 is associated with the host's excessive inflammatory response characterized by hyper-production of cytokines [4-6]. Preclinical and clinical studies have shown that macrolides regulate the inflammatory response, attenuating the production of anti-inflammatory cytokines and also promoting the production of immunoglobulins [7]. These regulatory effects on immune response reduce complications of respiratory viral infections [8-10]. Due to these immunomodulating properties, macrolides (eg. azithromycin, clarithromycin, erythromycin, fidaxomycin) have been studied extensively for their potential use as adjunctive broad spectrum therapy for viral respiratory infections including influenza [7, 10-13].

In this narrative review we will explore the role of macrolides in COVID-19 pathology, focusing on azithromycin, considering it the most suitable macrolide in a possible therapeutic combination.

We thus performed a literature search of MEDLINE with the following search terms "azithromycin and viral infections", "azithromycin and SARS-CoV2", "azithromycin and COVID-19", "azithromycin and Qt prolongation", "azithromycin and chloroquine and Qt prolongation". We have selected most updated evidences and all those relevant to synthesize the role of macrolides in COVID-19 treatment.

2. Macrolides in viral infections

Clarithromycin, azithromycin, erythromycin, bafilomycin A1 and telithromycin have shown to have anti-inflammatory and immunomodulatory effects [10]. For this reason, macrolides have been proposed as options for viral respiratory infections presenting an inflammatory basis, including COVID-19. Immunomodulating activities of azithromycin are explicated in two different moment of the disease, during the acute phase and at the resolution of the chronic inflammation. In the acute phase, the ability of azithromycin to reduce the production of pro-inflammatory cytokines such as IL-8, IL-6, TNF alpha, MMPs is thoroughly demonstrated [14]. In the resolution phase, this macrolide has been shown to increase neutrophil apoptosis and the oxidative stress related with inflammation. Also, clarithromycin, Bafilomycin A1 and Erythromycin has been found to inhibit the production of the intercellular adhesion molecule (ICAM)-1 and IL-1β, IL-6, IL-8 and TNF-α in rhinovirus and influenza infection models [11, 15-17]. Furthermore, in a study conducted by Murphy et al, azithromycin was associated with a shift of the T-helper phenotype from type I to type II, favoring tissue repair after the inflammation. Moreover, azithromycin attenuates the effects of lipopolysaccharide on lung allograft bronchial epithelial cells [11, 18-22].

In addition, this drug is able to significantly reduce the expression of iNOS and the pro-inflammatory macrophage receptor (CCR7) by increasing the activity of arginase and the anti-inflammatory macrophage receptors (MR and CD23) [23-25]. All these effects are explained by the azithromycin-mediated inhibition of the nuclear factor-kappa B (NF-kB).

Azithromycin has shown in vitro efficacy against Zika virus, reducing viral viability and proliferation of the virus [26] . Furthermore, a paper by Menzel et al. has demonstrated that azithromycin can transiently though strongly induce interferon expression in bronchial epithelium of patients with COPD when infected with rhinovirus [27] and this may explain the ability of azithromycin to reduce exacerbations frequency in COPD patients [28, 29].

Despite their well-established anti-inflammatory and immunomodulatory properties, macrolides do not have a direct antiviral effect and in clinical trials macrolides have shown controversial results. In one RCT, adult patients hospitalized for laboratory-confirmed flu were randomized to receive oseltamivir and azithromycin or oseltamivir alone, both for 5 days. Proinflammatory cytokines decreased more rapidly in the oseltamivir-azithromycin group. However, the decline in viral RNA was not affected by the addition of azithromycin [8]. In another prospective, double-blind, controlled trial in 24 healthy subjects inoculated with rhinovirus (who were seronegative for antibodies prior to the inoculation), assigned to receive either clarithromycin or trimethoprim-sulfamethoxazole, no effects have been noticed in favor of clarithromycin in terms of symptoms reduction or numbers of white blood cells and neutrophils, and in the concentrations of interleukins 6 and 8 in nasal lavage fluid during the cold [30].

In the study by Arabi et al., out of 349 patients with MERS in critical condition, 136 (39%) received macrolide therapy. Azithromycin was the most commonly used (97/136; 71.3%). Macrolide therapy was commonly started before the patient arrived in the intensive care unit (ICU) (51/136; 37.5%), or on day 1 in the ICU (53/136; 39%). At the time of ICU admission, the baseline characteristics of patients who received and did not received macrolides were similar, including demographics, and the sequential organ failure assessment score. Moreover, no statistically significant differences between in-hospital mortality, ICU and 90-day mortality, and hospital length of stay between the two groups were found [31].

In children hospitalized for respiratory syncytial virus (RSV) bronchiolitis, clarithromycin was associated with a reduction in hospital length of stay, oxygen need, treatment with β2-agonists, and re-hospitalizations within six months [12]. We should point out that this study has been widely criticized because of errors in statistical analysis, methodology and number of patients enrolled [32]. Results on hospital readmissions reported by Tahan et al. have not been confirmed in a larger RCT comparing azithromycin and placebo in children with bronchiolitis [33].

Azithromycin has proven to have an IC_{50} of 2,12 μM against SARS-CoV-2 in an in vitro screening of FDA approved chemical libraries [34].

In addition to the aforementioned effect on inflammatory response, macrolides could play a prophylactical role in pneumococcal and staphylococcal bacterial complications that occur with a certain frequency as complications of respiratory viral infections.

3. Efficacy of azithromycin in COVID-19

In a French clinical trial of 20 patients treated with hydroxychloroquine compared with 16 controls (patients who were refusing treatment with hydroxychloroquine or had a contraindication), six were treated with a combination of hydroxychloroquine 200 mg three times a day for ten days and azithromycin 500 mg on the first day, followed by 250 mg daily for other four days. Comparing the outcomes between patients treated with hydroxy-chloroquine alone, in combination with azithromycin or controls, the authors founded that 100% of patients treated with the combination were virologically healed at day 6 vs 57.1% of patients treated with only hydroxychloroquine and 12.5% of the control group ($p<0.05$) [35].

In contrast with this surprising result, Molina et al. reported the outcomes obtained in 11 consecutive patients treated with the combination of hydroxychloroquine plus azithromycin at the same dose scheme reported by Gautret et al.: none of the 11 patients had benefited from the treatment [35]. Of note, in the case series reported by Molina, 8 out of 11 patients did have significant comorbidities linked with poor outcomes (obesity, solid and hematological cancer, HIV- infection). One patient was discontinued after 4 days because of Qt prolongation.

An update of the study by Gautret reported a favorable outcome (defined as patient discharged not requiring aggressive oxygen therapy) in 65 out of 80 patients (81.3%) treated with hydroxychloroquine and azithromycin and a negative viral load test at 6 days in the 83% of patients with the combination. 15% required oxygen therapy, three needed an ICU admission but then improved and returned to the infectious disease ward, and only one died [35].

Two large studies on the efficacy of the combination of azithromycin and hydroxychloroquine were very recently published. Rosenberg et al. published a retrospective multicenter cohort study on 1438 hospitalized patients with COVID-19, 735 of which received hydroxychloroquine plus azithromycin as treatment for COVID-19. Comparing in-hospital mortality of patients receiving the combination, with that of subjects receiving hydroxychloroquine alone, azithromycin alone or no treatment, no significant differences were observed among the four groups [36]. Also, Mehra et al. reported an outcome against the benefit of the use of the association of hydroxychloroquine (or chloroquine) with a macrolide (azithromycin or clarithromycin) on a population of 96 032 patients hospitalized for COVID-19. The authors compared in-hospital mortality of patients treated with the combination macrolide/ quinoline derivatives with those of patients receiving no treatments for COVID-19: they found that combinations where associated with an increased risk of mortality [37].

Currently, many ongoing trials are evaluating efficacy of azithromycin in COVID-19. Azithromycin versus placebo, in combination or versus hydroxychloroquine or in triple combination with tocilizumab are the schemes predominantly evaluated (NCT04329832, NCT04341870, NCT04334382, NCT04348474, NCT04332107, NCT04341207, NCT04339426, NCT04329572, NCT04336332, NCT04332094,

NCT04335552, NCT04339816, NCT04338698, NCT04328272, NCT04347512, NCT04349592, NCT04345861, NCT04321278, NCT04344444, NCT04322396, NCT04322123, NCT04324463, NCT04334512, NCT04351919, NCT04341727, NCT04345419, NCT04332835, NCT04347031, NCT04349410).

A French trial is also evaluating the efficacy of azithromycin and hydroxychloroquine in the prevention of SARS-CoV-2 infection in health workers exposed to the virus (NCT04344379).

Azithromycin is also one of the drugs included in the large adaptive RECOVERY trial, the English national study sponsored by the University of Oxford EudraCT 2020-001113-21.

4. Co-administration of azithromycin and hydroxychloroquine and prolongation of the QT interval

Following some reports, in 2012 the FDA noticed a small increase in cardiovascular deaths and deaths from any cause among patients taking ~~a~~ azithromycin for a 5-day cycle course [38]. It was hypothesized that azithromycin could increase the Qtc with the risk of arrhythmias. On March 12, 2013, the FDA published a communication on azithromycin safety on heart rhythms warning on the risk of potentially fatal outcomes[39]. Following the revision of many studies both before and after the–public health note, the FDA modified it, stating that the potential risk must be assessed when azithromycin is used in the presence of risk factors such as QTc interval prolongation, hypokalemia, hypomagnesaemia, bradycardia or co-administration with antiarrhythmic drugs such as quinidine, procainamide, dofetilide, amiodarone and sotalol (drugs associated with prolongation at the QTc interval). In addition, it must be specified that the FDA note was linked to the reporting of torsade de pointes following the use of azithromycin in 12 patients (out of a few million treatments) who had at least two other risk factors each for torsade de pointes.

The association of azithromycin with QTc prolongation is controversial and still debated. Preclinical electrophysiological studies have extensively shown that azithromycin does not lengthen the Qtc. Azithromycin seems to have a rather low affinity for the hERG channel: at a high concentration of 300 mM, an inhibition of 22.5% of the hERG current was reported, with an IC50 value of 1091 mM, a concentration absolutely unattainable at doses used in humans. In addition, intravenous administration of azithromycin failed to produce a significant prolongation of the QTc interval in dogs with chronic atrioventricular block and there was also no increase in short-term variability (from beat to beat) in the potential repolarization of the monophasic action [40]. Furthermore, azithromycin has been used long-term in patients with COPD or cystic fibrosis without reports of cardiovascular death [28, 41].

Numerous other clinical studies have shown that Qtc prolongation following azithromycin administration is clinically irrelevant [42-44], but many others reported higher cardiovascular deaths [44-46] and cardiac arrhythmias, especially in the elderly population [47, 48]. A meta-analysis of 33 observational studies on 22,601,032 patients found a statistically significant increase in myocardial infarction risk associated with macrolides use (OR = 1.15 [95% CI, 1.01 to 1.30]), but authors noted that erythromycin and clarithromycin were associated with a higher risk, compared to azithromycin (OR = 1.58 [95% CI, 1.18 to 2.11] versus OR = 1.41 [95% CI, 1.11 to 1.81] respectively) [49].

4. Chloroquine/hydroxychloroquine azithromycin interaction

The proposed mechanism of QT prolongation induced by drugs is virtually the same for all medications. It is caused by a block in the outward IKr current, which is mediated by the potassium channel encoded by the KCNH2 gene. The reversibility with the drug discontinuation can be associated with a modification in extracellular potassium concentrations [50]. For this synergistic mechanism, co-prescription of QT prolonging medications is associated with a higher mortality rate [51].

Some studies have been carried out ~~on~~ assessing the risk of QT prolongation and fatal arrhythmias associated with the concomitant use of azithromycin and hydroxychloroquine, especially in patients with malaria.

Since alterations in the duration of cardiac action potential are a measure of cardiac instability associated with new onset of ventricular fibrillation, some authors have evaluated this parameter in guinea pigs. Pigs were anesthetized after the administration of azithromycin alone, chloroquine alone or the combination, reaching drugs plasma concentrations clinically used to manage malaria. Chloroquine alone produced a marked increase in the duration of action potential, and azithromycin did not. Azithromycin alone or in combination with chloroquine did not increase the action potential beyond the basic chloroquine responses, with no additional responsibility for arrhythmia [52].

In 2012, Pfizer, in order to use combination therapy to protect pregnant patients against malaria and sexually transmitted infections, conducted a randomized, placebo-controlled parallel study in 116 healthy controls who received 1000 mg of chloroquine alone or in combination with increasing doses of azithromycin (500, 1000 and 1500 mg). Concomitant administration of chloroquine with azithromycin increased the QTc interval ~~of~~ by 5, 7 and 9 ms, respectively [53]. There was a very close correlation between the azithromycin dose and the increase in the QTc interval.

In the following years, various studies have tested the combination of azithromycin-chloroquine or hydroxychloroquine in patients with malaria, with no reports of cardiovascular death [54, 55]. The azithromycin-chloroquine or hydroxychloroquine combination is currently in use in Africa, India, and Thailand for the treatment of malaria. More recently, a study on the use of Mobile Cardiac Outpatient Telemetry (MCOT) to monitor Qtc prolongation and any occurring arrhythmias was carried out. The authors reported that out of 28 the urgent alerts received by the cardiologist (by 18 patients out of 117) 5 alerts were for Qtc prolongation. In only one case hydroxychloroquine needed to be discontinued because of Qt prolongation [56]. In addition, 34.2% of patients was on treatment with at least another QT prolonging medication.

On the other hand, in a recent study on COVID-19 comparing safety and efficacy of chloroquine at high-dosage, 600mg twice daily for 10 days, versus chloroquine at low-dosage 450 mg twice daily on day 1 and once daily for 4 days, all patients were receiving azithromycin in association [57]. 11 out of 73 (15.1%) experienced QTc > 500 ms, with 8 out of 57 (14.0%) COVID-19 confirmed cases. QTc prolongation has been found more frequently in the high-dosage group 18.9% versus 11.1% in the low-dosage group. Two patients in the high-dose group experienced ventricular tachycardia before death but torsade de pointes were absent. Of note, 90% of patients were on treatment also with oseltamivir which is known to increase Qtc too. Also, previously mentioned studies by Rosenberg et al. and Mehra et al. respectively found a greater proportion of patients who experienced cardiac arrest (15.5%) and abnormal ECG findings (27.1%) among those receiving hydroxychloroquine plus azithromycin compared to hydroxychloroquine alone (13.7%and 27.3, respectively) and azithromycin alone (6.2% and 16.1%, respectively) and neither drug (6.8% and 14.0%, respectively) [36].-An increased risk of de-novo ventricular arrhythmia during hospitalization correlated with the use of hydroxychloroquine or chloroquine with a macrolide (8·1%; 5·106 HR, 95% CI 4·106–5·983; 6·5%; 4·011, 3·344–4·812 respectively) [37].

In addition, an evaluation of the Food and Drug Administration's Adverse Event Reporting System (FAERS) from 1969 to Q3/2019, using a disproportionality analysis method, revealed that hydroxychloroquine/chloroquine alone were not associated with an increase in safety signal, while azithromycin alone or in combination with hydroxychloroquine/chloroquine was associated with an increase in safety signal [58].

5. Precautions for the clinical use of the combination

Data from the literature on the risk of QT increase show that this side effect occurs in particular populations: long QT syndrome, bradycardias [59], arrhythmias, female sex [60], advanced age [59], patients with electrolyte imbalance and/or with pre-existing cardiac pathologies. Hypokalemia seems to be one of the major triggers [61].

Since the potential risk for QT prolongation was reported, both the American College of Cardiology [62] and the European Society of Cardiology [63] have defined recommendations for the use of azithromycin and hydroxychloroquine in combination ~~in~~ for COVID-19.

In patients, especially the elderly, who start a combination therapy with azithromycin and hydroxychloroquine the following steps are recommended:

- Careful evaluation of the patient's clinical features;
- Correction of hypokalemia to a level >4 mEq/l and of hypomagnesemia to a level of >2 mg/dl;
- Discontinuation of any therapy with proton pump inhibitors [64] (with the exclusion of patients with a documented history of ulcer~~,~~ or Zollinger-Ellison syndrome): they notoriously reduce the absorption of potassium and magnesium. To control a possible rebound in the production of hydrochloric acid that occurs with the suspension of PPI, the use antacid drugs (for example sucralfate) is suggested, being careful to distance their intake of at least 3 hours from COVID-19 therapies.

6. Conclusions

There are some promising evidences regarding the use of Azithromycin as a potential treatment for COVID-19, but more structured studies should be carried out. On the other hand, the benefit-risk assessment must be performed cautiously due to the potential cardiac harm that the association of azithromycin and hydroxychloroquine could cause, especially in more fragile patients, such as the elderly, patients with history of cardiovascular disease or comedications known to prolong Qtc. In particular, some measures must be implemented to provide patients safety.

Declarations

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