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Clinical evidences on the antiviral properties of macrolide antibiotics in the COVID-19 era and beyond

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Abstract

Macrolides are a large group of antibiotics characterised by the presence of a macro-lactone ring of variable size. The prototype of macrolide antibiotics, erythromycin was first produced by *Streptomyces* and associated species more than half a century ago; other related drugs were developed. These drugs have been shown to have several pharmacological properties: in addition to their antibiotic activity, they possess some anti-inflammatory properties and have been also considered against non-bacterial infections. In this review, we analysed the available clinical evidences regarding the potential anti-viral activity of macrolides, by focusing on erythromycin, clarithromycin and azithromycin. Overall, there is no significant evidences so far that macrolides might have a direct benefit on most of viral infections considered in this review (RSV, Influenza, coronaviruses, Ebola and Zika viruses). However, their clinical benefit cannot be ruled out without further and focused clinical studies. Macrolides may improve the clinical course of viral respiratory infections somehow, at least through indirect mechanisms relying on some and variable anti-inflammatory and/or immunomodulatory effects, in addition to their well-known antibacterial activity.

Keywords

Macrolides, erythromycin, azithromycin, clarithromycin, respiratory syncytial virus, influenza, coronavirus, SARS-CoV-2

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Introduction

General chemical structure of macrolides

Macrolides are a large class of antibiotics characterized by a macro-lactone ring of variable size (12–22 carbon atoms), connected to amino sugar and/or neutral sugar moieties by a glycosylic bond. Only those with 14, 15, or 16 membered lactone rings possess important therapeutic properties.[1] Initially, macrolides were produced naturally from *Streptomyces spp*. in the 1950s, but newer macrolides (including macrocycles and cyclic peptides) have been synthesized.[2] The prototype of macrolides is the 14-membered ring erythromycin (ERY), which has a broad-spectrum antibacterial activity by inhibiting protein synthesis in the target organism. It shows both bacteriostatic and bactericidal activities that depend on the concentration and the susceptibility of the target organism.[3] Clarithromycin (CLA) is another 14-membered macrolide, which has largely substituted ERY in the clinical practice. The only 15-membered ring is azithromycin (AZI), which is a derivative that belongs to the macrolide subclass, named azalides.[4] It is thought to have a broad-spectrum of antibacterial activity against gram-positive and gram-negative organisms, as well as atypical pathogens.[1] There are several 16-membered ring macrolides, which are both natural and synthetic drugs.[1,4]

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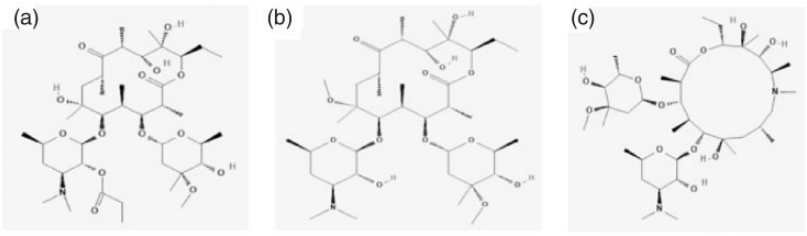

Figure 1. Chemical structures of 14-membered ring macrolides erythromycin(a), clarithromycin (b), and 15- membered ring azithromycin (c) sourced from the National Center for Biotechnology Information [PubChem Database: www.pubchem.ncbi.nlm.nih.gov, accessed on July 25, 2020].

Pharmacodynamic aspects of macrolides

As mentioned above, the most commonly prescribed macrolides in the clinical practice are AZI, CLA and ERY, whose chemical structure is showed in Figure 1. The latter two are metabolized in the liver by interacting with cytochrome P450 CYP3A4, which have been associated with different drug interactions. They are effective against several microorganisms and, depending on the concentration and the specific bacterial susceptibility, they show bacteriostatic and bactericidal activities. The main pharmacodynamic target is the bacterial ribosome, which is considered as one of the most conserved and complex cellular machines. Bacterial ribosomes are composed of two unequal subunits (small, 30S; large, 50S), which join together to form a 70S ribosome that is primed for the elongation phase of protein synthesis. Consequently, macrolides achieve their antibacterial activity by inhibiting the bacterial protein synthesis, through binding reversibly to the 50S subunit of the 70S bacterial ribosomes, which results in blocking any further protein translation.[1,5]

Pharmacokinetic aspects of macrolides

Macrolides are well tolerated antibiotics with an excellent safety profile, also in pregnant women and children. However, they are characterized by a poor digestive absorption, which was estimated to be $<60\%$, and significantly influenced by food. They exhibit good tissue distribution in general, except for the cerebrospinal fluid. Moreover, they may have a few adverse events, mostly gastrointestinal (e.g. nausea, vomiting and rarely abdominal pain); however, all macrolides are associated with QTc interval prolongation, which can become clinically relevant in specific patients and comorbid clinical situations.[6,7]

Generally, macrolides accumulate in the intracellular compartment, mostly in the macrophages and the polymorphonuclear leukocytes.[3,5] However, there are significant differences among the different macrolides in terms of their drug half-life ($T_{1/2}$) and peak serum concentrations (C_{max}),, as summarized in Table 1.[1,5] Also, several reports claimed significant differences among different pharmaceutical formulations of the same macrolide molecule: for example, in the treatment of streptococcal pharyngitis, ERY estolate was shown to be effective at lower doses than ERY ethyl-succinate.[8,9] Furthermore, a study by Croteau et al. comparing the ERY estolate and ERY ethyl-succinate in human volunteers, showed that the base generated from the former have higher bioavailability than that generated from the latter.[10]

Table 1. Pharmacokinetic parameters of the three main macrolide antibiotics.

Macrolide	C_{max} (mg/L)	$T_{1/2}$ (h)
Erythromycin	1.5	2
Azithromycin	0.4	4.7
Clarithromycin	2.1	4.7

Clinical indications of macrolide antibiotics

The clinical use of ERY has been reduced after the introduction of the second generation of 14-membered macrolides (including CLA), due to the improved pharmacokinetic properties of these hemi-synthesis derivates. The third main molecule of this class is AZI: the introduction of a nitrogen atom in this 15-membered macrolide, has been shown to increase its intracellular penetration, especially in macrophages and neutrophils.[1]

These macrolide antibiotics (ERY, CLA, AZI) are currently used against a large spectrum of bacteria and, thus, are indicated to treat several types of human infectious diseases, both in adults and children. Indeed, macrolides are active against gram-positive bacteria (including *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*; however, they are not effective against most *enterococcus spp.*). They are considered less effective against gram-negative bacteria, even though the structural peculiarities of AZI increased the antibiotic activity in this specific regard. These macrolides are considered

among the first-line treatments for several specific infections sustained by gram-negative microorganisms, like *Bordetella pertussis*, *Campylobacter spp.* and *Helicobacter pylori*. Importantly, they are used against many "intra-cellular" bacteria, including *Mycoplasma pneumoniae*, *Bartonella henselae* and *Legionella spp.*[11–13]

In general, in addition to these specific infections, macrolides are mainly used to treat upper and lower respiratory infections and, therefore, are particularly valuable in paediatric patients, for whom other classes of antibiotics (e.g. tetracyclines and fluoroquinolones) are not recommended or even contraindicated. In detail, macrolides in children are the safest alternative if there is established or suspected allergy to beta-lactams antibiotics and the first-line treatment against *Mycoplasma pneumoniae*, which is responsible of a consistent part of lower respiratory infections (including pneumonia) in children and adolescents and may trigger a wide range of immune-mediated extra-pulmonary manifestations.[14–16]

Additional properties of macrolide antibiotics

Macrolides have been demonstrated to possess some anti-inflammatory activity both *in vitro* and *in vivo*. Indeed, macrolides could modulate the cytokine production as well as other immunological cellular properties (e.g. chemotaxis, degranulation, oxidative burst, and even apoptosis). Some studies also suggested that these molecules can influence the mucus secretion and characteristics and affect some biological properties of lung epithelial cells by enhancing the inter-cellular tight junctions, for instance. These aspects are considered an additional value in the treatment of (infectious) inflammatory respiratory disorders and, thus, especially in those patients with underlying comorbidity (e.g. chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis, asthma).[1,17,18] Interestingly, because of these anti-inflammatory properties, macrolide antibiotics have been also investigated in the management of some autoimmune disorders (e.g. rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus).[19–21]

Importantly, as regards infectious diseases, the clinical relevance of macrolides may be beyond their antibacterial activity. For instance, these molecules have been also considered as anti-parasitic drugs: whereas the clinical efficacy of ERY against *Plasmodium spp.* was not established, AZI is currently investigated in clinical trials as both prophylactic and therapeutic antimalarial treatment for uncomplicated cases and in combination with other traditional antimalarial drugs. Indeed, AZI seems to inhibit the protein synthesis on the prokaryote-like ribosomes inside a plasmodial cellular organelle, known as apicoplast.[3,17]

Finally, macrolides have been empirically used in patients affected with a variety of viral diseases, in order to prevent any bacterial superimposition at least.[22–24] In this regard, macrolides have been speculated or suggested to have some direct beneficial effects for the treatment of viral infections.[25] In this review, we aim to summarize and discuss the main studies investigating and/or assessing the potential clinical role of macrolides on specific viral infections.

Macrolides in viral diseases

Respiratory syncytial virus

Respiratory Syncytial Virus (RSV) is a single-stranded RNA virus belonging to the "Paramyxoviridae" family: it causes respiratory infections and, in detail, is a leading cause of virus-induced lower respiratory tract disease among infants (e.g. bronchiolitis).[26]

The output of our literature research on the clinical use of macrolide antibiotics in the treatment of RSV infections consisted of four clinical double-blind, randomized and placebo-controlled trials only, as summarized in Table 2.[27–30] Additional clinical studies on the use of macrolides in bronchiolitis can be found, but they do not consider the specific etiological diagnosis by RSV. Among the aforementioned four RSV-specific studies, only Tahan F. et al. reported some benefit from CLA therapy, in terms of hospitalization length and duration of oxygen supplementation or other supportive therapies.[29] The remaining three trials used AZI and included more patients: overall, these studies did not show any significant advantage from the use of AZI, in terms of number of hospitalization days, symptoms duration and prevention of wheezing or other respiratory problems in the 4 months following this antibiotic therapy (which was specifically assessed by Beigelman et al).[27,28,30] However, in the study by Tahan F et al., the duration therapy was 3 weeks, thus, longer than the other three trials (Beigelman et al., Pinto et al.: $7+7$ days; Kneyber et al.: 3 days only) and, interestingly, the macrolide was CLA (vs. AZI used in the other trials).[27–30] Accordingly, two systematic reviews concluded that there was no evidence to support the use of antibiotics in treatment of bronchiolitis, in general.[31,32]

Therefore, the available evidences so far do not clearly support the use of macrolide antibiotics in children with RSV infections. However, additional and tailored studies may be needed to make any final conclusions. Indeed, recent experimental studies provided data supporting some potential and indirect beneficial effects of macrolides against RSV infection. Yamamoto K et al. examined the effect of CLA on the pro-inflammatory cytokine production (including

Table 2. Clinical studies assessing macrolide antibiotics in RSV positive respiratory infections.

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
Tahan F et al. (Turkey, 2007)[29]	CLA	Double-blind, monocentric randomized, placebo-controlled	Infants < 7 months of age with documented RSV positive respiratory tract infection, requiring inpatient care	21 (12 *vs.* 9)	Oral CLA (15 mg/kg/day) for 3 weeks *vs.* oral placebo	- CLA was associated with a significant reduction in the hospital length stay (51 h. *vs* 88 h., $p < 0.05$) - The duration of supplemental oxygen and intravenous fluids treatments was higher in the placebo group (31 hrs. *vs.* 72 hrs., $p < 0.05$; 26 h. *vs.* 56 h, $p < 0.05$, respectively.) - Significant difference in the use of bronchodilators: moreover, shorter period in the CLA group (5 *vs.* 7 days, $p < 0.05$).	- Significant decreases in the plasma IL-4, IL-8 and eotaxin levels following CLA therapy (however, the statistical significance of these results between CLA and placebo groups, is not clearly showed by the authors).
Kneyber MC et al. (Netherlands, 2008)[28]	AZI	Double-blind Multicentric, randomized, placebo-controlled	Patients < 24 months of age with a confirmed diagnosis of RSV low respiratory tract disease	71 (32 *vs.* 39)	Oral AZI (10 mg/kg/day) for 3 days *vs.* oral placebo	- No difference in the mean duration of hospitalization; - "AZI was not associated with a stronger resolution of clinical symptoms of disease severity compared to placebo or with a shorter duration of supportive therapy".	–
Pinto LA et al. (Brazil, 2012)[30]	AZI	Double-blind Multicentric, randomized, placebo-controlled	Infants < 12 months of age with documented RSV positive bronchiolitis	104 (47 *vs.* 57)	Oral AZI (10 mg/kg/day for 7 days, followed by 5 mg/kg/day for 7 days more) *vs.* oral placebo	- There was no beneficial effect (in terms of length of oxygen requirements and/or hospital stay) by treatment group in patients who had RSV infection, once stratified by age.	- Results for other secondary outcomes, such as antibiotic or bronchodilator prescriptions, also did not show any significant differences between groups.
Beigelman A et al. (2015, USA)[27]	AZI	Double-blind, monocentric randomized,	Infants (1–18 months) with RSV positive bronchiolitis,	39 (19 *vs.* 20)	Oral AZI (10 mg/kg/day for 7 days, followed by 5 mg/kg/day for	- No difference in the proportion of patients who experienced 2 or more wheezing episodes over	- AZI treatment did not result in any reduction in serum IL-8 levels by day 8;

(continued)

Table 2. Continued.

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
		placebo-controlled	requiring inpatient care		7 days more) *vs.* oral placebo	the 50 weeks after treatment; - significant reduction of days with respiratory symptoms (cough, wheeze, or shortness of breath) over the following 50 weeks (36.7 *vs.* 70.1 days, $p = 0.01$).	- AZI treatment showed a significant reduction in nasal lavage IL-8 level measured between day 1 and day 15 ($p = 0.026$), but not at day 8 or when assessed including all time points, overall.

interferons), by primary human nasal epithelial cells and lung epithelial cell lines, after infection by RSV. They found that CLA strongly suppressed the RSV-induced production of IFN-β and IFN-γ in their cellular experimental systems.[33] Yokota S et al. provided similar evidences in terms of immunomodulatory effects on the production of pro-inflammatory cytokines triggered by RSV; interestingly, they reported that CLA may also suppress the expression of platelet-activating factor (PAF) receptor in the pulmonary epithelial cell line A549, which is a receptor for *Streptococcus pneumoniae* and, thus, may promote bacterial superinfections following RSV disease.[34] Finally, Mosquera RA et al. recently reported that the prophylactic use of AZI reduced the airway inflammation and mortality in a RSV mouse infection model.[35] In summary, no direct and/or specific action of macrolide antibiotics against RSV can be currently demonstrated. However, some indirect effects may be plausible, with most of the data regarding CLA.

Influenza viruses

Influenza viruses belong to the family of RNA viruses termed "Orthomyxoviridae". They are divided into 3 main types: A, B, and C. Most of the epidemics and outbreaks of flu are caused by types A and B, whereas type C is generally responsible for sporadic mild upper respiratory symptoms.[36]

Some *in vitro* and animal experiments suggested the potential usefulness of macrolide antibiotics as part of a combination therapy during influenza. In 1998, Sato K et al. provided evidences on a mouse model that ERY can reduce the lung injury caused by influenza viruses, due to its anti-inflammatory properties.[37] A similar action was recently demonstrated for CLA as well: according to the experiments by Takahashi E et al., it modulates infection-related inflammation by suppressing the induction of MCP-1 and MMP-9.[38] The same research group also described that CLA can increase the secretory IgA production by upregulating the expression of BAFF molecules in mucosal dendritic cells of influenza A-infected mice.[39] Moreover, Namkoong H et al. reported that CLA can promote the expansion of a specific $CD11b^{+}Gr\text{-}1^{+}$ cell population, which seems to play a role in the immunomodulatory mechanisms of macrolides.[40]

However, CLA effects may go beyond the simple "anti-inflammatory effect". *In vitro* studies suggested that CLA may inhibit human influenza virus infection by reducing the expression of specific glycoproteins used by the virus to enter into the airway epithelial cells,[41] and may also negatively affect the viral replication cycle.[42] AZI was reported to interfere with virus internalization process in a study by by Tran DH

et al.[43] Some beneficial effects of CLA were recently reported in animal models (mice, monkeys) treated with combination therapies, including antiviral agent.[44,45] Conversely, Fage C et al. concluded that the inclusion of AZI in the combination therapy provided no additional clinical or antiviral benefits over oseltamivir monotherapy in their experimental murine models infected with influenza A.[46]

In summary, most experimental and clinical studies investigating the effects of macrolide antibiotics against influenza viruses (mostly type A) were focused on CLA. Even if potential direct and indirect mechanisms by which CLA may works against this virus have been reported, actually the clinical evidence is not strong enough to conclude for any significant medical effect of this antibiotic in flu patients, as summarized in Table 3.[47–55] Indeed, most clinical studies assessed CLA (and macrolides) in combination therapy with neuraminidase inhibitors (e.g. oseltamivir, zanamivir): the inclusion of CLA in the therapy of influenza-related pneumonia can definitely reduce the risk of bacterial superinfection and related complications, even though a direct antiviral effect has not been clearly defined, yet. However, it is worth to be emphasized the fact that CLA was able to boost the specific antibody responses against the virus in two studies.[47,49]

Coronaviruses

Coronaviruses (CoVs) are a group of single stranded RNA viruses, some which can cause zoonotic infections in humans. SARS-CoV caused an epidemic of severe pneumonia in 2002–2003, an epidemic of unusual cases of pneumonia with severe acute respiratory distress, which mainly affected China and South-East Asia. MERS-CoV caused an outbreak of low respiratory tract infections with respiratory distress in a few Arab countries (Saudi Arabia, Oman, UAE) in 2012, even though some cases were subsequently reported in 24 other countries outside the region. Currently, another coronavirus (SARS-CoV-2) have been causing a pandemic, as declared in March 2020 by the World Health Organization.[56]

Multiple therapeutic regimens against SARS-CoV-2 have been used in the last few months and some of them included also macrolide antibiotics and, in detail, AZI in combination with other drugs. These attempts mainly derived from some previous reports on the co-administration of macrolides during other respiratory infections in patients with pneumonia, especially if characterized by interstitial radiological patterns. Indeed, as showed in Table 4, the are no clear evidence of efficacy of macrolides against coronaviruses.[57–62]

Actually, most data supporting a potential use of macrolide antibiotics against CoVs derived from *in vitro* experiments, most of them not specifically related to this virus family.[63] Touret F et al. described the *in vitro* screening of 90 approved drugs that could have a potential antiviral activity: some of them, including AZI, were reported as the most promising.[64] Moreover, a bioinformatics analysis included AZI as a candidate drug against SARS-CoV-2, due to its biological property to inhibit autophagy, whose mechanisms may play a role in several viral infections.[65] Andreani J et al. described *in vitro* experiments suggesting a synergistic effect of AZI in combination with hydroxy-chloroquine against SARS-CoV-2, at concentrations that were compatible with those obtained in the human lungs by using therapeutic dosages.[66] Very recently, Ulrich H et al. speculated that AZI might prevent the viral cell invasion by interfering with the CD147 interactions, as it may happen with *Plasmodium falciparum* to enter into the red blood cells. Indeed, along with angiotensin-converting enzyme 2 (ACE2), CD147 (also known as EMMPRIN), has been recognized as a receptor exploited by SARS-CoV-2 to infect the host cells. However, such an effect may be partially indirect, due to the anti-inflammatory properties of macrolides, as CD147 expression is enhanced during the acute inflammation.[67]

Therefore, clear evidences of clinical efficacy of macrolide antibiotics against CoVs are currently lacking; the potential mechanisms are elusive and, at the moment, most of the benefit, if present, may derive by the anti-inflammatory and immune-modulatory properties of these molecules.

Ebola virus

Ebola virus is a member of Filoviridae family and is the causative agent of viral haemorrhagic fever, which is associated with a very high mortality rate in Africa.[68] While the management of Ebola virus consisted of supportive care measures such as fluid and electrolyte replacement, several investigational therapeutics were tested in clinical trials and animal models. Macrolides were one class of many drugs tested against Ebola virus activity both *in vivo* and *in vitro*. The drug showed *in vitro* anti-Ebola activity, but the results were inconclusive or mismatching in animal models.[69,70] Sun W et al. found two sets of three-drug combinations that significantly improved the efficacy of individual drugs against the Ebola virus infection *in vitro* at clinically relevant concentrations and one consisted of toremifene-CLA-posaconazole.[71] Du X et al., by combinatorial screening using pseudo-virion and mini-genome replicon systems, identified several drugs with some activity against Ebola virus and, among them, there

Table 3. Clinical studies assessing macrolide antibiotics in Influenza virus positive respiratory infections [OSV: oseltamivir; ZNV: zanamivir; NAI: neuraminidase inhibitor].

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
Sawabuchi T et al. (Japan, 2009)[47]	CLA	Retrospective observational study	Children with Influenza A infection	47	Oral CLA 5 mg/kg for 5 days: - CLA (8) - OSV+CLA (12) - OSV (14) - None (6)	- The frequency of residual cough in the OSV+CLA group was significantly lower than in the other groups, including the group treated with OSV.	- Significant increases in the levels of anti-viral sIgA were found in the CLA and OSV+CLA groups. The addition of CLA to OSV resulted to augment the sIgA production.
Ishii I et al. (Japan, 2012)[48]	CLA	Multicentric open-label prospective study	Adult outpatients with Influenza A infection	141 (74 vs. 27)	Oral CLA 400 mg/day for 5 days (+ NAI) ***vs.*** NAI alone	- There was no significant increase in the efficacy of treatment on the duration of disease signs/symptoms when CLA was added,	
Shinahara V et al. (Japan, 2013)[49]	CLA	Retrospective observational study	Children affected with Influenza A infection	195	Oral CLA at 5.0–7.5 mg/kg for 5 days OSV (70) ZNV (27) OSV+CLA (20) ZNV+CLA (10) None (68)	- no assessment of clinical parameters - However, the re-infection rates in the subsequent season were significantly higher in the OSV and ZNV groups than the untreated, while CAM+OSV and CAM+ZNV tended to reduce such rate.	- Treatment of influenza with OSV and ZNV for 5 days attenuated the induction of anti-viral S-IgA in nasal washes and anti-viral IgG in serum, compared with the untreated group. The combination of CLA with OSV or ZNV boosted and restored the production of mucosal S-IgA and systemic IgG.
Higashi F et al. (Japan, 2014)[51]	CLA	Monocentric randomized, prospective open-label study	Patients > 15 years with Influenza A and/or B infections	63 (31 vs. 32)	Oral CLA 400 mg/day (+ NAI) ***vs.*** AI alone	- Among all patients, fever duration was approximately 7 h (21%) shorter in the CLA group than the control group, but this difference was not statistically significant. Anyway, the duration of fever inpatients with body temperatures	- Among these patients, the improvement of rhinorrhea in the CLA group was higher than the control group (88% vs. 20%; $p = 0.03$). - Serum IL-6 levels decreased 5 days after treatment, but no

(continued)

Table 3. Continued.

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
						≧38.5 C at the start of treatment was approximately 42% shorter ($p = 0.02$) in the CLA group.	significant difference between the groups.
Kakeya H et al. (Japan, 2014)[50]	AZI	Monocentric randomized, prospective open-label study	Patients > 20 years with Influenza A and/or B infections	107 (57 vs. 51)	"Extended-release formulation of single-dose oral AZI 2,000 mg" (+ OSV) for 5 days ***vs.*** OSV alone	- A significant decrease in the maximum temperature was observed on day 4 with the combined therapy ($p = 0.037$). In addition, the maximum temperature on days 3 through 5 was significantly lower in the combo-group ($p = 0.048$). - No significant differences were observed between the 2 groups in the resolution time of the main influenza symptoms. However, the CLA group showed a trend toward earlier resolution of fever.	- Overall, statistically significant differences were not observed in the expression levels of inflammatory cytokines and chemokines between the 2 groups.
Hung IFN J et al. (Hong Kong, 2017)[53]	CLA	Multicentric, Randomized, open-label, controlled Phase IIb/III Trial	Adult patients hospitalized for A(H3N2) influenza	217 (107 vs. 110)	Oral CLA 500 mg/day for 5 days (+ OSV + naproxen) ***vs.*** OSV alone	- The combination treatment was associated with lower 30-day mortality ($p = 0.01$), less frequent ICU admission ($p = 0.009$), and shorter hospital stay ($p < 0.001$). - Multivariate analysis showed that combination treatment was the only independent factor associated with lower 30-day mortality rate (OR, 0.06; 95% CI, 0.004-0.94; $p = 0.04$).	- The virus titers (days 1–3; $p < 0.01$) in the nasopharyngeal NPA specimens were significantly lower in the CLA group.

(continued)

Table 3. Continued.

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
Yatera K et al. (Japan, 2017)[54]	CLA	Multicentric, open-label, prospective study	Adult outpatients with Influenza A	64 (38 vs. 26)	Oral CLA 400 mg/day for 5 days (+NAI) ***vs.*** NAI alone	- Overall, the CLA group showed a significantly shorter time to clear the fever than the control group, especially in patients with Influenza A infection (who were elderly or have comorbidities).	- the duration of cough was significantly longer in the CLA group than in the control group. The relatively higher rate of patients with asthma in the CLA group was claimed to explain the longer duration of cough.
Lee N et al. (Hong Kong, 2017)[52]	AZI	Randomized open-label multicenter trial	Patients > 18 years with Influenza A and/or B viruses	50 (25 *vs.* 25)	Oral AZI 500 mg for 5 days (+OSV) ***vs.***OSV alone	- No statistical differences between treatment groups in terms of complication rates, need of supplemental oxygen and assisted ventilation, and duration of hospitalization	- a significant anti-inflammatory effects (IL-6 and IL-8 levels) was observed in the CLA group with severe influenza infection. - no significant difference in viral RNA load change or culture-negativity by day 5 between groups.
Ishaqui AA et al. (Saudi Arabia, 2020)[55]	AZI	Retrospective observational cohort study	Hospitalized adult Patients with Influenza A infection	329 (102 *vs.* 227)	Oral/I.V. AZI 500 mg/day for 5 days (+ OSV) ***vs.*** OSV alone	- The AZI group was associated with shorter length of hospitalization (6.58 vs 5.09 days; $p < 0.0001$) and less frequent need of respiratory support (38.3% vs 17.6%; $p = 0.016$). - Overall the influenza symptoms severity score was significantly lower for the AZI group on day-5 of hospitalization.	-

Table 4. Clinical studies assessing macrolide antibiotics in coronaviruses positive respiratory infections [HCQ: hydroxychloroquine; ICU: intensive care unit].

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
Zhao Z et al. (China, 2003)[57]	AZI	Monocentric retrospective cohort study	Adult patients with SARS	190	4 groups (3 of them including AZI I.V. 400–600 mg/day) variably associated with IFN-α, other antibiotics and steroids	- Comparing the clinical outcomes of the different therapies, the group therapy, including levofloxacin 200 mg b.i.d. plus azithromycin 600 mg/day, provided the best results.	- Early and aggressive use of steroids combined with non-invasive ventilatory support offered the best hope for a favorable outcome
Arabi YM et al. (Saudi Arabia, 2019)[58]	AZI CLA ERY	Multicentric retrospective cohort study	ICU Adult Patients with MERS	349 (136 vs. 213)	3 groups: AZI (n = 97) CLA (n = 28) ERY (n = 22) ***vs.*** No Macrolide (213)	- no statistically significant differences between ICU and hospital mortality, hospital length of stay between the 'macrolide therapy' groups and 'no macrolide therapy' group.	- macrolide therapy was not associated with difference in viral clearance
Gautret P et al. (France, 2020)[59]	AZI	Observational, non-randomized, open-label*	Patients > 12 years with COVID-19	36* [6 patients received AZI in addition to HCQ]	Oral AZI (500 mg on day-1 followed by 250 mg per day, the next four days)	- At day-6, 100% of patients treated with hydroxychloroquine and AZI combination cleared the virus, compared to 57.1% in patients treated with HCQ only, and 12.5% in the control group ($p < 0.001$).	–
Gautret P et al. (France, 2020)[60]	AZI	Observational single arm study	Patients > 12 years with COVID-19	80	Oral AZI (500 mg on day-1 followed by 250 mg per day, the next four days)	- 81.3% of patients had favorable outcome and were discharged from the general ward. Only 15% required oxygen therapy and three patients were transferred to the ICU. Only one patient (86 years old) died in the Infectious Diseases ward.	- A rapid decrease of nasopharyngeal viral load tested by PCR was noted, with 83% negative at Day-7, and 93% at Day-8.
Magagnoli J et al. (USA, 2020)[62]	AZI	Monocentric Retrospective cohort study	Adult Patients with COVID-19	368	HCQ = 97 HCQ+AZI = 113 None = 158 [no dosage information provided]	- No evidence that the use of HCQ, either with or without AZI, could reduce the risk of mechanical ventilation in patients hospitalized with Covid-19. An	–

(continued)

Table 4. Continued.

Authorship (year, country)	Macrolide	Study design	Study population	Patients' number	Intervention	Main clinical outcome	Additional findings
						association of increased overall mortality was identified in patients treated with HCQ.	
Molina JM et al. (France, 2020)[61]	AZI	Observational single arm study	Adult Patients with COVID-19	11	Oral AZI (500 mg on day-1, followed by 250 mg/day for the four days.	- "Within 5 days, one patient died, two were transferred to the ICU".	- Repeated nasopharyngeal swabs in 10 patients using PCR assay were still positive for SARS-CoV2 RNA in 8/10 patients at day-5 after treatment initiation.

was AZI.[72] While it is not yet known whether the macrolide antibiotic has a direct antiviral inhibitory effect, it might be speculated that some antiviral effect may be due its ability to amplify systemic antiviral response mediated by the IFN pathway.[73]

Zika virus

Zika virus is an arbovirus belonging to the Flaviviridae family. The infection is transmitted to humans via mosquito vectors and usually causes flu-like symptoms. Moreover, it is reported as associated with severe neurological complications in adults and, when the infection occurs in pregnant women, the virus can also pass to the fetus and cause central nervous system malformations (e.g. microcephaly). Currently there is no vaccine or antiviral treatment against Zika virus infections.[74,75]

A number of different therapeutics were tested for their potential effect against the virus. One of the tested drugs is AZI, which showed inhibitory effects *in vitro* and in some animal models. A recent study by Li C et al. suggested that AZI can effectively suppressed viral infection *in vitro*, which they claimed to work by targeting a late stage in the viral replication cycle. They also proposed that the drug protects against the virus by improving antiviral immunity through upregulating the expression of some viral induced pathogen recognition receptors (PRRs).[76] However, Retallack H et al. showed that AZI could reduce the viral proliferation and virus-induced cytopathic effects in glial cell lines and human astrocytes.[77] Very recently, Wang X et al. described their *in vitro* and *in vivo* (mouse model) data supporting a significant anti-Zika virus activity of ERY, which would interfere with the viral entry into the cell.[78]

Therefore, macrolides might deserve some clinical studies to assess their potential usefulness against Zika virus, as proposed by Iannetta et al.[79]

Conclusions

Overall, there is no significant and/or clinically relevant evidence so far that macrolides might have a direct benefit on most of viral infections considered in this review. However, their clinical benefit cannot be ruled out and they may improve the clinical course of viral respiratory infections (RSV, Influenza viruses and CoVs) at least through indirect mechanisms relying on some and variable anti-inflammatory activity. Interestingly, some authors reported a boost activity of CLA on the antibody response against influenza. Recent experimental data also suggested a potential role of macrolides against the infections caused by arboviruses, especially Zika virus, but no clinical

trials are available at the moment. In general, further and focused clinical studies are needed to assess the effective contribution and potential mechanisms of macrolides in the management of specific viral infections.

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