Pharmacology & Therapeutics 143 (2014) 225–245

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

# Azithromycin: Mechanisms of action and their relevance for clinical applications

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ARTICLE INFO

Available online 11 March 2014

*Keywords:*
Azithromycin
Macrolide antibiotic
Mechanisms of action
Pharmacokinetics
Immunomodulation
Clinical efficacy

ABSTRACT

Azithromycin is a macrolide antibiotic which inhibits bacterial protein synthesis, quorum-sensing and reduces the formation of biofilm. Accumulating effectively in cells, particularly phagocytes, it is delivered in high concentrations to sites of infection, as reflected in rapid plasma clearance and extensive tissue distribution. Azithromycin is indicated for respiratory, urogenital, dermal and other bacterial infections, and exerts immunomodulatory effects in chronic inflammatory disorders, including diffuse panbronchiolitis, post-transplant bronchiolitis and rosacea. Modulation of host responses facilitates its long-term therapeutic benefit in cystic fibrosis, non-cystic fibrosis bronchiectasis, exacerbations of chronic obstructive pulmonary disease (COPD) and non-eosinophilic asthma.

Initial, stimulatory effects of azithromycin on immune and epithelial cells, involving interactions with phospholipids and Erk1/2, are followed by later modulation of transcription factors AP-1, NFκB, inflammatory cytokine and mucin release. Delayed inhibitory effects on cell function and high lysosomal accumulation accompany disruption of protein and intracellular lipid transport, regulation of surface receptor expression, of macrophage phenotype and autophagy. These later changes underlie many immunomodulatory effects of azithromycin, contributing to resolution of acute infections and reduction of exacerbations in chronic airway diseases. A sub-group of post-transplant bronchiolitis patients appears to be sensitive to azithromycin, as may be patients with severe sepsis. Other promising indications include chronic prostatitis and periodontitis, but weak activity in malaria is unlikely to prove crucial. Long-term administration of azithromycin must be balanced against the potential for increased bacterial resistance. Azithromycin has a very good record of safety, but recent reports indicate rare cases of cardiac *torsades des pointes* in patients at risk.



*Abbreviations:* AECOPD, acute exacerbations of COPD; AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; AUC, area under the plasma concentration-versus-time curve; BAL, broncho-alveolar lavage; BOS, bronchiolitis obliterans syndrome; CAD, cationic amphiphilic drugs; CAP, community acquired pneumonia; CBP, chronic bacterial prostatitis; CF, cystic fibrosis; CFTR, CF transmembrane conduction regulatory protein; $C_{max}$, peak plasma concentration; COPD, chronic obstructive pulmonary disease; COX, cyclo-oxygenase; cPLA2, cytoplasmic phospholipase A2; DNFB, dinitrofluorobenzene; DPB, diffuse panbronchiolitis; DTH, delayed type hypersensitivity; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GM-CSF, granulocyte–macrophage colony stimulating factor; GVH, graft versus host reaction; HR, hazard ratio; IL, interleukin; i.n, intranasal; i.p, intraperitoneal; i.t, intratracheal; i.v, intravenous; JNK, c-Jun NH(2)-terminal kinase; LC3, microtubule-associated protein 1A/light chain 3; LOS, length of stay; LPS, bacterial lipopolysaccharide; LRTI, lower respiratory tract infection; MAPK, mitogen-activated protein kinase; MDR1, multidrug resistance protein 1; MIC, minimum inhibitory concentration; MLSbK, macrolides, lincosamines, streptogramin B and ketolides; MMP, metalloproteinase; MODS, multiple organ dysfunction syndrome; MPO, myeloperoxidase; MUC5AC, mucin 5AC; MV, mechanical ventilation; NFκB, nuclear factor kappaB; NGU, non-gonococcal urethritis; NK, natural killer cells; NR, not reported; OR, odds ratio; p.o, oral; PG, prostaglandin; PI, phosphatidylinositol; PI3K, phosphoinositide-3-kinase; PK, pharmacokinetic; PMNL, polymorphonuclear leukocytes; PS, phosphatidylserine; RCT, randomized controlled clinical trial; ROS, reactive oxygen species; SAA, serum amyloid A protein; SMC, smooth muscle cell; SREBP, sterol regulatory element binding protein; STAT, signal transducers and activators of transcription; STD, sexually transmitted disease; t1/2, plasma half-life; TLR, Toll-like receptor; $T_{max}$, time to $C_{max}$; TNFα, tumor necrosis factor alpha; Th, T helper cell; VAP, ventilation assisted pneumonia; $V_d$, volume of distribution; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.pharmthera.2014.03.003
0163-7258/

**Contents**



## 1. Introduction

Azithromycin, a second generation macrolide, broad-spectrum antibacterial, has received increasing attention in recent years because of additional effects on host-defence reactions and chronic human diseases. It is the prototype 15-membered lactone ring azalide, synthesized in the early 1980s as a semi-synthetic derivative of erythromycin. Discovered around the same time by researchers at Pfizer in the United States (Bright & Hauske, 1984) and at PLIVA, Croatia (Kobrehel et al., 1982), PLIVA patented first and licensed the compound to Pfizer. With much improved pharmacokinetic properties over erythromycin, azithromycin became the most widely used broad-spectrum antibacterial in North America. Pfizer Inc's Arthur E. Girard and Gene Michael Bright, together with PLIVA's Slobodan Djokic (posthumously) and Gabrijela Kobrehel, received in 2000 the American Chemical Society's award of "Heroes of Chemistry who have promoted human welfare in the area of health" for their discovery of Zithromax® (azithromycin).

Azithromycin shares the same mechanism of antibacterial action as other macrolide antibiotics (Allen, 2002), but accumulates more effectively in phagocytes, thus being delivered in high concentrations to sites of infection (Miossec-Bartoli et al., 1999; Wilms et al., 2006). It also inhibits bacterial quorum-sensing and reduces formation of biofilm and mucus production, which extend its range of antibacterial actions (Tateda et al., 2001; Hoffmann et al., 2007). As an antibiotic, azithromycin is indicated for respiratory, urogenital, dermal and other bacterial infections, but has beneficial effects in chronic inflammatory disorders such as diffuse panbronchiolits, bronchiolitis obliterans and rosacea. Efficacy in these conditions is ascribed to immunomodulatory effects on innate and adaptive immune responses. Modulation of host response reactions also accounts, at least partially, for beneficial effects in cystic fibrosis, non-cystic fibrosis bronchiectasis, bronchial obliterans syndrome (BOS) and chronic obstructive pulmonary disease (COPD).

Azithromycin is well-tolerated and has a very good record of safety. Although macrolides have a class warning for potential cardiac QT prolongation, azithromycin does not show this effect under experimental conditions (Milberg et al., 2002). Until recently, only a handful of cases of QT prolongation had been reported for patients treated with the drug (Kezerashvili et al., 2007). This is mainly because azithromycin, unlike other macrolide antibiotics, does not interact with CYP3A4, despite a minor interaction with the anti-coagulant warfarin (Kanoh & Rubin, 2010; Mergenhagen et al., 2013). Recently, evidence for increased risk of QT prolongation with azithromycin has appeared, but mainly in patients with greater susceptibility to adverse cardiac effects (Giudicessi & Ackerman, 2013).

We review here the antibacterial actions and pharmacokinetics of azithromycin, as well as its immunomodulatory effects and the mechanisms involved, in comparison to other macrolide antibiotics. We discuss the main clinical uses of azithromycin, drawing attention to emerging indications and emphasising how its pharmacokinetic properties and immunomodulatory actions contribute to the effects observed.

## 2. Antibacterial mechanisms of action

### 2.1. Inhibitory actions

Azithromycin, like other macrolide antibiotics, inhibits bacterial protein synthesis by binding to and interfering with the assembly of the 50S large ribosomal subunit and the growth of the nascent polypeptide chain (Champney & Burdine, 1998; Champney et al., 1998; Hansen et al., 2002). It binds at the polypeptide exit tunnel, close to the peptidyl transferase center (PTC) on the 23S rRNA, but does not inhibit PT activity, in contrast to larger macrocyclic antibiotics. The basicity of azithromycin leads to faster penetration of the outer membranes and more effective entrance into the bacteria, thereby enhancing activity against Gram-negative bacteria (Dinos et al., 2001).

Binding sites on the bacterial ribosome for the structurally different macrolides, lincosamines, streptogramin B and ketolides (MLSbK) overlap significantly, so that changes in a single ribosomal region simultaneously alter susceptibility to various MLSbK antibiotics.

Although ineffective as a bactericidal agent against *Pseudomonas aeruginosa* at clinically relevant concentrations, azithromycin inhibits the generation of both growth-stimulating, quorum-sensing compounds, and alginate biofilm which protects the micro-organism from antibiotic actions (Tateda et al., 2001; Hoffmann et al., 2007). Efficacy against both *P. aeruginosa* virulence factor production and biofilm formation, as well as its ability to decrease the minimum inhibitory concentration (MIC) of anti-pseudomonas agents (Lutz et al., 2012) are related to ribosomal protein synthesis inhibition (Kohler et al., 2007).

Azithromycin also shows moderate activity against the malaria parasite, *Plasmodium* spp., exhibited as delayed killing, which is achieved via the apicoplast (Dahl & Rosenthal, 2007). Clinical studies, however, failed to demonstrate even equivalence of three-day treatment with azithromycin to other antimalarials or drug combinations (van Eijk & Terlouw, 2011).

### 2.2. Resistance phenotypes

Bacteria resist azithromycin in two ways: a) by changing the target/binding site via methylation of key rRNA nucleotides or mutation of some ribosomal components and b) by efflux pump activity, thereby decreasing its intrabacterial accumulation.

Ribosome methylation is the most important resistance mechanism for all MLSbK antibiotics (Sutcliffe & Leclerq, 2002). The MLSbK-II phenotype, characterized by the presence of *Erm* methylases, is highly resistant to all MLSbK antibiotics, including azithromycin, which induces *erm* genes in *Streptococci* and in *Staphylococci* (Sutcliffe &