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Evaluation of macrolides for possible use against multidrug-resistant *Mycobacterium tuberculosis*

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ABSTRACT Multidrug-resistant tuberculosis (MDR-TB) is a major global health problem. The loss of susceptibility to an increasing number of drugs behoves us to consider the evaluation of non-traditional anti-tuberculosis drugs.

Clarithromycin, a macrolide antibiotic, is defined as a group 5 anti-tuberculosis drug by the World Health Organization; however, its role or efficacy in the treatment of MDR-TB is unclear. A systematic review of the literature was conducted to summarise the evidence for the activity of macrolides against MDR-TB, by evaluating *in vitro, in vivo* and clinical studies. PubMed and Embase were searched for English language articles up to May 2014.

Even though high minimum inhibitory concentration values are usually found, suggesting low activity against *Mycobacterium tuberculosis*, the potential benefits of macrolides are their accumulation in the relevant compartments and cells in the lungs, their immunomodulatory effects and their synergistic activity with other anti-TB drugs.

A future perspective may be use of more potent macrolide analogues to enhance the activity of the treatment regimen.

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Introduction

Multidrug-resistant tuberculosis (MDR-TB) still remains a major problem in global health. MDR-TB is caused by *Mycobacterium tuberculosis* that is resistant to at least isoniazid and rifampicin [1–3], depriving the patient of the two most effective drugs used. MDR-TB should be treated by a regimen containing at least four anti-tuberculosis (TB) drugs to which the bacterium is susceptible [4]. By the end of 2012 it was estimated that globally 3.7% of treatment-naïve TB patients and 20% of previously treated cases have MDR-TB [5], in 2013 this number was adjusted to 3.5% and 20.5%, respectively [6]. The problem of drug resistant *M. tuberculosis* is increasing, both in terms of loss of susceptibility to an increasing number of drugs, as well as in terms of the proportion and absolute numbers of TB patients with MDR-TB [7–9]. Evaluation of the effectiveness of non-traditional or newly synthesised anti-tubercular drugs is therefore necessary. Clarithromycin (CLR), a macrolide, is classified as a "group 5" anti-TB drug, defined by the World Health Organization (WHO) as anti-TB drugs with unclear efficacy or an unclear role in the treatment of MDR-TB [4]. Macrolides are a group of antibiotics already widely used for the treatment of upper and lower respiratory tract infections. The molecular structure consists of a macrocyclic lactone ring with various amino sugar side groups. The macrolides in clinical use have a 14-, 15- and 16-membered lactone ring. They can be subdivided into natural products (erythromycin) and semi-synthetic derivatives (CLR, roxithromycin and ketolides) [10, 11]. Macrolides are considered bacteriostatic agents in clinically useful concentrations and act by inhibiting protein synthesis through binding to the large 50S ribosomal subunit [2, 12].

Some semi-synthetic macrolides have already shown efficacy against, and are part of standard therapy against, many nontuberculous mycobacteria [13], such as *Mycobacterium leprae* [14–16], *Mycobacterium ulcerans* [17, 18] and *Mycobacterium avium* [19, 20]. As stated in the WHO guidelines, little is known about the effectiveness of macrolides against *M. tuberculosis* [4]. We, therefore, conducted a systematic review of the literature to summarise the evidence for the activity of macrolides against MDR-TB, by evaluating *in vitro*, *in vivo* and clinical studies.

Mechanism of action

Macrolides, azalides and ketolides exert antibacterial activity by inhibiting protein synthesis through binding to the 50S ribosomal subunit near the peptidyl transferase centre in *Deinococcus radiodurans* [21, 22] and *Mycobacterium smegmatis* [23]. They block the exit tunnel through which newly synthesised peptides move away from the peptidyl transferase centre, obstructing elongation of the peptide chain. It is thought that a second inhibitory activity blocks the assembly of rRNAs and r-proteins resulting in inhibition of formation of the large 50S ribosomal subunit [24, 25]. It is not clear whether these mechanisms of action are the same for slowly growing mycobacteria as for *M. smegmatis*. One study showed that CLR disorganises the outer wall layer and cytoplasmic membranes in the *M. avium* cell envelope eventually leading to bacterial death [26]. Furthermore, it is thought that macrolides tend to exert immunomodulatory effects in respiratory diseases, independent of their antimicrobial effects [27]. The putative immunomodulatory mechanisms of macrolides include improvement of the primary defence reaction, decreasing neutrophil migration and infiltration into respiratory epithelium, downregulation of the inflammatory cascade, reduction in generation of oxygen-free radicals, modulation of chemokine release and decreasing excessive pro-inflammatory mediators and cytokine production. These effects are hypothesised to control exacerbations of various chronic respiratory diseases, like cystic fibrosis, chronic obstructive pulmonary disease, asthma, chronic bronchitis and diffuse panbronchiolitis, and probably contribute to the therapeutic effect. Furthermore, it has been shown that macrolides reduce airway hyperresponsiveness and improve pulmonary function [28–34].

Methods

Articles concerning macrolide activity and/or efficacy against multidrug-resistant *M. tuberculosis* retrieved using a specific search strategy, described in the following section, were reviewed.

Search strategy

To retrieve relevant articles that describe the activity of macrolides against multidrug-resistant *M. tuberculosis*, the following search terms were used to search in PubMed: ("Tuberculosis"[Mesh] OR tubercul*[tw]) AND ("Tuberculosis, Multidrug-Resistant"[Mesh] OR MDR*[tw] OR resist*[tw]) AND ("Macrolides"[Mesh] OR "telithromycin" [Supplementary Concept] OR "cethromycin" [Supplementary Concept] OR "CEM 101" [Supplementary Concept] OR erythromycin*[tw] OR telithromycin*[tw] OR cethromycin*[tw] OR Clarithromycin*[tw] OR Azithromycin*[tw] OR Roxithromycin*[tw]OR telithromycin*[tw] OR solithromycin*[tw]OR cethromycin*[tw]). In Embase, the same search term was inserted as follows: "tuberculosis"/exp OR tubercul*:ab,ti OR "multidrug resistant tuberculosis"/exp AND (mdr*:ab,ti OR resist*:de,ab,ti) AND ("macrolide"/exp OR "ketolide"/exp OR erythromycin*:ab,ti OR clarithromycin*:ab,ti OR azithromycin*:ab,ti OR roxithromycin*:ab,ti OR telithromycin*:ab,ti OR

solithromycin*:ab,ti OR cethromycin*:ab,ti). Relevant articles were included up until May 2014 and supplemented by hand-searching the reference lists of identified eligible studies. Articles described as editorials, comments, reviews or case studies were not included. Citations were screened independently by more than one reviewer, examining titles and abstracts to identify potentially relevant articles. Differences were resolved by consensus. Extracted data from the searches were entered into Excel (Microsoft, Redmond, WA, USA), duplicates were removed and original English language articles were then retrieved and the full text screened for final inclusion and data extraction. No attempts were made to obtain missing data from the researchers of eligible studies.

Selection criteria

Studies were eligible for inclusion if the activity of a specific macrolide against multidrug resistant *M. tuberculosis* was evaluated in *in vitro*, *in vivo* or clinical studies. *In vitro* studies were included if the method of testing susceptibility of MDR-TB to macrolides was adequately described, including the radiometric BACTEC method (Becton Dickinson Microbiologic Systems, Detroit, MI, USA), microplate Alamar blue assay (MABA) (Accumed International, Westlake, OH, USA), proportion methods, and broth dilution and microdilution methods in different media. *In vitro* studies were subsequently subdivided into studies that evaluated extracellular and intracellular susceptibility, and drug–drug synergy. As data on *in vitro* intracellular susceptibility testing of MDR-TB strains was lacking, data from experiments with drug-susceptible strains were included.

The susceptibility of MDR-TB to anti-TB drugs is described by the minimum inhibitory concentration (MIC). The MIC is the lowest concentration that prevents growth of bacteria (visually or photometrically determined). MIC_{90} is the MIC that inhibits 90% of the isolates or strains. A MIC range of 2–4 $\mu g \cdot mL^{-1}$ is defined as being a low MIC value, 8–16 $\mu g \cdot mL^{-1}$ as intermediate and $\geqslant 16$ $\mu g \cdot mL^{-1}$ as a high MIC value. In addition, studies are included that present data on susceptibility as viable count enumeration (VCE) (*i.e.* bactericidal activity of a drug) or number of colony-forming units (CFU) [35].

To assess whether macrolides show synergy, indifference or antagonism with other anti-TB drugs, studies describing the combination of a macrolide and any drug used for the treatment of MDR-TB as defined by the WHO treatment guidelines were included. Often the fractional inhibitory concentration (FIC) is used to describe the interaction between two antimicrobial agents. The FIC is determined as follows:

$$FIC_A = (MIC_{A,\ combination}/MIC_{A,\ alone}) + (MIC_{B,\ combination}/MIC_{B,\ alone})$$

where FIC_A is the FIC of drug A, $MIC_{A,\ combination}$ is the MIC of drug A when combined with a second drug, $MIC_{A,\ alone}$ is the MIC of drug A when used alone, $MIC_{B,\ combination}$ is the MIC of drug B when combined with a second drug, $MIC_{B,\ alone}$ is the MIC of drug B when used alone. The FIC was interpreted as follows: FIC $\leqslant 0.5$ suggests synergistic activity, FIC 1–4 suggests indifference and FIC >4 suggests antagonism [36, 37].

In vivo animal studies were included if adequate data were provided on measures of effect in comparison with control (mean $\log_{10}$ CFUs in spleen and lungs, mortality, and weight of spleen), type of TB strain, type of animal strain, type of infection model, drug doses, treatment duration and organs studied. In addition, if synergism between two drugs was described, information or data on the difference between the mean $\log_{10}$ CFUs of the combination of drugs and the lowest mean $\log_{10}$ CFU of one drug used alone had to be reported.

Clinical studies reporting on treatment of MDR-TB using macrolides were included if they at least reported data on the treatment regimen (including CLR) and treatment outcome (efficacy and/or adverse events) or pharmacokinetics.

Results

In total 1713 articles were retrieved from the initial search (fig. 1). After screening of titles and abstracts, only 169 articles were considered eligible for evaluation. Ultimately, 37 articles were included describing *in vitro* susceptibility testing of macrolides against MDR-TB strains, *in vitro* synergy testing with any drugs for the treatment of MDR-TB as defined by the WHO treatment guidelines, *in vivo* studies and clinical studies.

In vitro *studies*

Extracellular susceptibility testing

Table 1 presents the results for studies concerning extracellular susceptibility testing. Most studies performed drug susceptibility testing against *M. tuberculosis* by determining the MIC using the radiometric BACTEC 460 method [36–41, 44–46]. Often a concentration range up to 16 $\mu g \cdot mL^{-1}$ was used [36, 37, 39, 41]. Overall, studies have shown no susceptibility of MDR-TB strains to CLR, with all studies reporting

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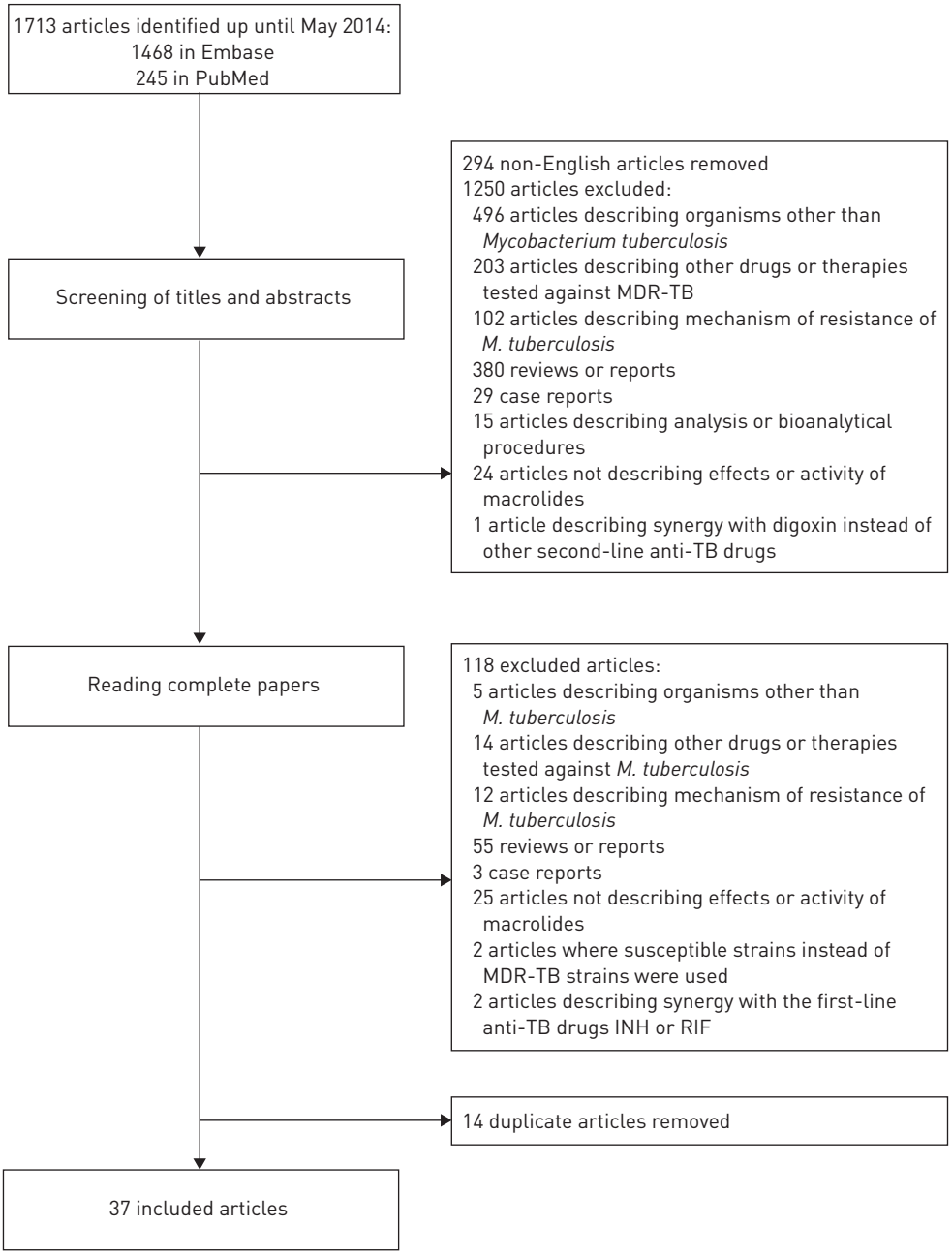

FIGURE 1 Flow chart of the selection procedure. MDR-TB: multidrug-resistant tuberculosis; TB: tuberculosis; INH: isoniazid; RIF: rifampicin.

high MIC values [36–41]. This was also observed for roxithromycin, azithromycin (AZI) [41] and the metabolite of CLR 14-hydroxyclarithromycin [36]. However, in another study the MIC values for CLR against 69 MDR-TB isolates were <4 $\mu g \cdot mL^{-1}$, with only one strain being resistant [46].

In addition, other studies have reported rather high MICs obtained using the proportion method with Middlebrook 7H10 medium. MICs in these studies also indicate that CLR [43] and roxithromycin [45] are unsuitable alternative agents when used alone against MDR-TB, based on high MIC_{90} and MIC values, respectively. CLR, erythromycin and AZI were also shown to be inactive against 17 MDR-TB isolates when using the broth dilution method in 7H9 Middlebrook medium [42]. The broth dilution and microdilution method were also used in 7HSF medium, which resulted in MICs for CLR of 20 $\mu g \cdot mL^{-1}$ [48] and 25 $\mu g \cdot mL^{-1}$ [49]. Furthermore, the use of the MABA with three MDR-TB strains resulted in MIC values in the range of 2–32 $\mu g \cdot mL^{-1}$. When tested against the virulent H37Rv strain ROX, AZI and telithromycin

TABLE 1 Extracellular *in vitro* susceptibility testing

First author [ref.]	Strain	Strains n	Method	Concentration range $\mu g \cdot mL^{-1}$	Drugs	pH	MIC $\mu g \cdot mL^{-1}$	MIC_{90} $\mu g \cdot mL^{-1}$	Mean log_{10} reduction in viable counts
Bergmann [38]	CI, MDR-TB	5	BAC	1.0–8.0	CLR		>8		
Cavalieri [36]	CI, MDR-TB	6	BAC	0.25–16	CLR		⩾16		
					14-OH-CLR		>16		
Luna-Herrera [39]	CI, MDR-TB	5	BAC	0.06–16.0	CLR		4–⩾16		
Stoffels [37]	CI, MDR-TB	16	BAC	2, 4, 8, 16	CLR		⩽2–>16		
Bosne-David [40]	CI, MDR-TB	14	BAC	4	CLR		>4		
Hoffner [41]	CI, MDR-TB	17	BAC	2, 4, 8, 16	CLR		2–⩾16		
					ROX		4–⩾16		
					AZI		>16		
Yew [42]	CI, MDR-TB	17	BM-7H9	0.03–8.0	CLR		0.25–>8.0	>8	
					ERY		>8	>8	
					AZI		4.0–>8.0	>8	
Lu [43]	CI, MDR-TB	22	PM	2–128	CLR			64	
Rastogi [44]	CI, MDR-TB	3	BAC	5–20	CLR	6.8	10–20		
						7.4	4–16		
		2	VCE	40	CLR				<1
Rastogi [45]	CI, MDR-TB	13	BAC	0.5, 1, 2, 4, 8, 16, 32, 64	ROX	6.8	32–>64		
						7.4	16–>32		
			PM	0.5, 1, 2, 4, 8, 16, 32, 64		6.8	64–>64		
	CI, MDR-TB	1	VCE	64		6.8			1
				32		7.4			1–2
Umubyeyi [46]	CI, MDR-TB	69	BAC	4	CLR		<4		
Falzari [47]	H37Rv	1	MABA	3–104	CLR		5.98		
					ROX		26.79		
					AZI		95.87		
					CETH		3.06		
					TELI		103.93		
	CI, MDR-TB	3			CLR		2.0–32.0		
Sato [48]	Kurono MDR-TB	1	BM-7HSF	0.5–128	CLR		20		
Tomioka [49]	Kurono MDR-TB	1	BMM	?–25[#]	CLR		25		

MIC: minimal inhibitory concentration; MIC_{90}: MIC that inhibits 90% of the isolates or strains; CI: clinical isolate; MDR-TB: multidrug-resistant tuberculosis; BAC: Radiometric BACTEC TB 460; CLR: clarithromycin; 14-OH-CLR: 14-hydroxyclarithromycin; ROX: roxithromycin; AZI: azithromycin; BM-7H9: broth dilution method with 7H9 medium; ERY: erythromycin; PM: proportion method with Middlebrook 7H10 agar; VCE: viable count enumeration; MABA: microplate Alamar blue assay; CETH: cethromycin; TELI: telithromycin; BM-7HSF: broth dilution method with 7HSF medium; BMM: broth microdilution method using 7SHF medium. [#]: tested range described in the methods section.

resulted in high MIC values of 6–96 $\mu g \cdot mL^{-1}$, while CLR gave a MIC of ~6 $\mu g \cdot mL^{-1}$ and cethromycin yielded a lower MIC of 3 $\mu g \cdot mL^{-1}$ [47].

Some studies using the BACTEC method or proportion method did not report their results in terms of MICs, but as the number of strains that were resistant or susceptible to a macrolide. Different studies have reported 11 (84.6%) out of 13 [50], 19 (63.3%) out of 30 [51], six (24%) out of 25 [52] or eight (10.3%) out of 78 [53] MDR-TB strains as being resistant to CLR using the radiometric BACTEC method. Another study reported that five (6.9%) out of 72 MDR-TB strains were resistant to CLR/AZI as estimated using the BACTEC or proportion method [54]. However, using the proportion method 52 (51.5%) out of 101 [55] and 517 (88.1%) out of 587 [56] clinical isolates from MDR-TB patients were susceptible to CLR. Furthermore, in this study four (50%) out of eight clinical isolates from extensively drug-resistant TB (XDR-TB) patients were susceptible to CLR [56].

Some studies have addressed the influence of pH on *in vitro* susceptibility testing using the radiometric BACTEC method. MICs of CLR and roxithromycin appeared to be lower at pH 7.4 compared with the routine pH of 6.8 [44, 45]. This was also seen when the bacterial viability was determined by VCE; roxithromycin appeared to be more active at pH 7.4 compared with pH 6.8 [45].

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Irrespective of the methods used, all studies show high MIC values, suggesting low susceptibility of MDR-TB to macrolides.

Intracellular susceptibility testing

Results for intracellular susceptibility testing are presented in table 2. Most studies assessed the intracellular susceptibility of bacteria to macrolides by counting CFU or MIC determination in *M. tuberculosis*-infected macrophages, alveolar cells and alveolar macrophages [39, 44, 47–49]. A dose-dependent inhibition of growth was observed for CLR against MDR-TB, H37Rv and two clinical isolates in J774A.1 mouse macrophages, with a 2–3 log reduced growth [39]. CLR and cethromycin showed a more modest bacteriostatic effect against drug-susceptible strains in J774A.1 mouse macrophages [47] and human macrophages [44], with a reduction of <1 $\log_{10}$ CFU. Furthermore, CLR displayed a modest inhibition of growth of the Kurono MDR-TB strain in Mono-Mac-6 human monocytic cell lines (MM6) and A549 type-II human alveolar epithelial cell lines (A549-II) [48, 49]. In the same study, MICs of 5 and 2.5 $\mu g \cdot mL^{-1}$ were determined in MM6 and A549-II, respectively [48].

Thus, overall CLR resulted in the reduction of intracellular CFU counts.

Synergy testing

Results for synergy testing are presented in table 3. The combination of CLR and rifabutin resulted in a FIC of 1–2, suggesting indifference [38]. When CLR was combined with ethambutol (EMB) a synergistic effect was observed [36, 40]. CLR and AZI also acted synergistically with spectinomycin against the drug susceptible H37Rv strain, FICs were all $\leqslant$0.5. When tested within macrophages CLR showed synergy with spectinomycin, but AZI did not [58]. Finally, synergy was also observed between CLR and linezolid (LZD) with FICs in the range of 0.32–0.37 and 0.375–1.0 using the mycobacteria growth indicator tube 960 system (MGIT) (Becton Dickinson) and the absolute concentration method, respectively [57].

Thus CLR shows promising synergistic results when combined with other second line anti-TB drugs.

In vivo *studies*

Results from *in vivo* studies are presented in table 4. In murine infection models CLR showed modest activity, resulting in inhibition of growth of *M. tuberculosis* and MDR-TB. CLR reduced CFU counts in spleens and lungs compared with those of mice given no CLR treatment [47, 59]. CLR showed less activity against organisms in the lungs than organisms in the spleens. In another study, CLR showed complete protection from death since none of the CLR treated mice in the first experiment died during 8 weeks of treatment, whereas all non-treated control mice died within the same period. Furthermore, in the second arm of this study, in which controls did survive up to 4 weeks, CLR reduced mean CFU counts in both lungs and spleens compared with controls [39]. Also, CLR was given in combination with streptomycin or

TABLE 2 Intracellular *in vitro* susceptibility testing

First author [ref.]	Strains	Cells	Method	Concentration range	Drugs	Cells	MIC	$\Delta\log_{10}$ CFU reduction[#]
Luna-Herrera [39]	2 CI, MDR-TB	J774A.1 murine macrophages	GI	0.06–2.0 $\mu g \cdot mL^{-1}$	CLR			2–3[¶]
	2 CI							2–3[¶]
	1 H37Rv							2[¶]
Rastogi [44]	H37Rv	PBMC-derived human macrophages	CFU counts	4 $\mu g \cdot mL^{-1}$	CLR			<1
Falzari [47]	Erdman	J774A.1 murine macrophages	CFU counts	0.06, 0.32, 1.6, 8 μM	CLR			<1
					CETH			<1
Tomioka [49]	Kurono	MM6	CFU counts	2.3 $\mu g \cdot mL^{-1}$	CLR	MM6		1–2
	MDR-TB	A549-II				A549		1–2
Sato [48]	Kurono	MM6	CFU counts	2.3 $\mu g \cdot mL^{-1}$	CLR	MM6		1–2
	MDR-TB	A549-II				A549		2
			MIC determination			MM6	5 $\mu g \cdot mL^{-1}$	
						A549	2.5 $\mu g \cdot mL^{-1}$	

MIC: minimal inhibitory concentration; CI: clinical isolate; MDR-TB: multidrug-resistant tuberculosis; J774A.1: murine monocyte macrophage cell line; GI: growth index; CLR: clarithromycin; PBMC: peripheral blood monocytic cells; CETH: cethromycin; MM6: Mono-Mac-6 human monocytic cell line; A549-II: type II human alveolar epithelial cell lines. [#]: results are read from graphs, therefore a certain error must be taken into account; [¶]: not a $\log_{10}$ CFU reduction but $\log_{10}$ reduced growth measured using growth index.

TABLE 3 *In vitro* synergy of clarithromycin (CLR) and other second-line tuberculosis drugs

First author [ref.]	Strain	Strains n	Drugs	Concentration range	FIC
Bergmann [38]	CI, MDR-TB	5	CLR Rifabutin	1, 0.5, 0.25, 0.125 × MIC	1.00–2.00
Cavalieri [36]	CI, MDR-TB	6	CLR 14-OH-CLR	2.0 μg·mL^{-1} 0.5 μg·mL^{-1}	1.125–1.250
Cavalieri [36]	CI, MDR-TB	5	CLR:14-OH-CLR (4:1) EMB	MIC of each drug and at four twofold dilutions lower	0.06–0.13
Bosne-David [40]	CI, MDR-TB	14	CLR EMB	2.0 μg·mL^{-1} 1.2, 1.8, 2.5, 5 μg·mL^{-1}	0.05–0.49[#]
Bolhuis [57]	CI, MDR-TB	13	CLR Linezolid	0–8 μg·mL^{-1} 0–0.5 μg·mL^{-1}	0.32–0.37 (MGIT) 0.375–1.0 (ACM)
Ramón-García [58]	H37Rv		CLR and Spectinomycin AZI and Spectinomycin	0.25 × MIC	<0.51 0.31–0.5
	M. tuberculosis infected macrophages		CLR and Spectinomycin AZI and Spectinomycin		0.25–0.5 1

FIC: fractional inhibitory concentration; CI: clinical isolate; MDR-TB: multidrug-resistant tuberculosis; MIC: minimal inhibitory concentration; 14-OH-CLR: 14-hydroxyclarithromycin; EMB: ethambutol; MGIT: Mycobacteria Growth indicator tube (MGIT) 960 system; ACM: absolute concentration method; AZI: azithromycin; *M. tuberculosis*: *Mycobacterium tuberculosis*. [#]: not measured by FIC but by X/Y quotient.

thioacetazone. No significant difference in CFU was observed in mice treated with streptomycin alone or with a combination of streptomycin and CLR. However, activity of the combination of thioacetazone plus CLR was slightly better than when the drugs were given separately, with CLR having slightly better activity than thioacetazone. In another study, mortality rate decreased by 60.8% and a 125 mg difference in spleen weight was observed when CLR treated mice were compared with controls [60]. CFU counts in this study were difficult to compare, since the high mortality rate in the control group led to grossly underestimated CFU counts.

Overall CLR reduces mortality and provides protection from death when used in TB-infected mice, reducing mean CFU counts.

Clinical studies

Data on the clinical use of macrolides for the treatment of MDR-TB is scarce. Most studies evaluating MDR-TB treatment do not include patients with CLR or do not present the individual data. One study described the influence of CLR on LZD exposure in five MDR-TB patients. While 250 mg CLR once daily had no statistically significant effect, 500 mg of CLR significantly increased LZD serum exposure by a median of 44% compared with baseline [61]. Another retrospective study reported that when CLR was included in a salvage regimen, this did not necessarily result in improved culture conversion [62]. However, it is difficult to estimate the impact of the data from this study since only 14 patients with a complex medical history received CLR in their salvage regimen.

A number of studies also reported inclusion of macrolides in a treatment regimen for MDR- or XDR-TB, where some studies also included observed adverse events. The frequency with which macrolides were prescribed varied, depending on the type of resistance (MDR-TB or XDR-TB). One meta-analysis shows that macrolides given in the clinic do not result in superior treatment outcomes compared with other group 5 drugs [63]. Three (0.6%) out of 481 MDR-TB patients received CLR over a period from 1992–2002 in South Africa [64]. 121 (11.6%) out of 1047 MDR-TB patients from Latvia, Lima, Manila and Tomsk received CLR in their treatment regimen [65]. 72 (34.1%) out of 211 MDR-TB patients received CLR and 15 (7.1%) out of 211 received roxithromycin as part of their treatment [66]. In four out of 13 patients CLR was used over a period of 3.6 years [67]. Among 655 subjects evaluated, CLR was prescribed 44 times and roxithromycin once, furthermore CLR was discontinued twice (4.5%) due to adverse drug reactions of the gastrointestinal tract and headache [68]. 28 (10.6%) out of 263 MDR-TB patients received CLR in an anti-TB regimen, where CLR was withdrawn in two patients due to severe side-effects [69]. One (5.9%) patient discontinued treatment with CLR due to nephrotoxicity out of

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TABLE 4 *In vivo* results of treatment with macrolides

First author [ref.]	Strain	Mice	Infection	Drug	Dose	Treatment duration	End-point	Organs	Log_{10} CFU	Result
Klemens [59]	1 MDR-TB CI	Female outbred 4-week-old CD-1 mice	Intravenous	CLR	200 mg·kg^{-1} 5 days·week^{-1}	4 weeks	CFU counts	Spleen	−1.63	
								Lungs	−0.97	
Luna-Herrera [39]	H37Rv	Either sex 4–6-week-old C57BL/6 mice	Intravenous	CLR	200 mg·kg^{-1} 5 days per week	4 weeks	CFU counts	Spleen	−0.69	
						8 weeks			NA	
						4 weeks		Lung	−1.41	
						8 weeks			NA	
				CLR+TZ		4 weeks		Spleen	−0.16	
						8 weeks			−0.31	
						4 weeks		Lung	−0.95	
						8 weeks			−0.29	
				CLR+SM		4 weeks		Spleen	−0.29	
						8 weeks			+0.01	
						4 weeks		Lung	−0.02	
						8 weeks			+0.37	
							Mortality rate			−100%
Truffot-Pernot [60]	H37Rv	Female Swiss mice	Intravenous	CLR	200 mg·kg^{-1} 6 times per week	6 weeks (spleen)	Mortality rate			−60.8%
						4 weeks (lung)	Spleen weight			−125 mg
Falzari [47]	Erdman	Female 8-week-old BALB/c mice	Aerosol	CLR	200 mg·kg^{-1} daily	20 days	CFU counts	Lungs	−0.94	

MDR-TB: multidrug-resistant tuberculosis; CI: clinical isolate; CLR: clarithromycin; NA: not applicable; TZ: thioacetazoe; SM: streptomycin.

17 patients given CLR treatment [51]. One (4%) out of 25 received treatment with CLR over a period of 2 years in Madrid [52]. Furthermore, another article assessed the treatment outcome in relation to inclusion of CLR in the previous TB regimen. Six (21.4%) out of 28 patients with a favourable outcome had CLR in their previous TB regimen, as opposed to 21 (18.4%) out of 114 patients with a poor outcome having CLR in their previous TB regimen. Cure and treatment completion was defined as a favourable outcome and treatment failure, death and default were defined as a poor outcome. Finally, in a study among UK children with MDR-TB, in three out of the 17 children evaluated CLR was included in the regimen [70].

Macrolide use appears more frequent in patients with XDR-TB. 21 (44.6%) out of 47 XDR-TB patients received CLR, with a treatment duration of 14.5 (11.6–18.9) months [71]. Another study reports that 23 (53.5%) out of 43 XDR-TB patients received CLR and six (14.0%) out of 43 received roxithromycin in their treatment regimen [66]. Furthermore, 11 (9.6%) out of 115 XDR-TB patients received AZI and 77 (67%) out of 115 XDR-TB patients received CLR, no severe adverse events were observed for both drugs [72].

These studies demonstrate that macrolides are included treatment regimens when strains become more drug-resistant (MDR-TB and XDR-TB), when other drug treatment options fail.

Discussion

Macrolides have high MIC values in *M. tuberculosis* isolates when evaluated *in vitro* [36–45, 47–49]. However, some studies reported promising results that might contribute to clinical activity in the sense of macrolides exerting bacteriostatic effects *in vitro* [39, 45, 48, 49] and *in vivo* [39, 47, 59]. Furthermore, a decrease in mortality in murine infection models [39, 60] and synergistic effects when CLR was combined *in vitro* with second-line anti-TB drugs [36, 37, 40, 57, 73] were seen. Also, an interaction was reported in a clinical study [61], suggesting an increase of LZD serum concentration when combined with CLR.

Despite high MIC values, some bronchopulmonary pharmacokinetic studies demonstrated that high concentrations of CLR were achieved in alveolar macrophages and epithelial lining fluid (ELF), with high ratios of concentrations in ELF and alveolar macrophages compared with that in plasma assessed in healthy volunteers [74–76]. These results suggest that high concentrations of CLR are achieved in ELF and alveolar macrophage cells, which exceed the *in vitro* MIC values by several fold. Especially in alveolar macrophages, the concentrations of CLR reached may exceed MIC values that could help eradication of MDR-TB. Therefore, the high MIC values obtained from extracellular *in vitro* studies may not necessarily indicate the full potential efficacy of CLR against MDR-TB, since this drug is not equally distributed in the body but accumulates in relevant target compartments and target cells in the lungs. This should be considered when giving the drug to a patient with extrapulmonary TB. In intracellular susceptibility

studies CLR resulted in reduction of CFU counts [39, 48, 49]. This effect can be explained by other studies describing the ability of CLR to accumulate in phagocytic cells, resulting in high intracellular to extracellular concentration ratios [77, 78].

An important aspect seen for CLR is the synergistic effect when it is combined with other anti-TB drugs. This synergistic effect may be substance-dependent with a preference towards drugs with a smaller molecular volume, like EMB. If CLR affects the outer cell wall layer and cytoplasmic membrane of *M. tuberculosis,* as it does in *M. avium* [26], it is possible that the permeability for other drugs increases, resulting in a synergistic effect. Unfortunately no data is available on combination with kanamycin, amikacin, moxifloxacin and levofloxacin. For this reason future prospects for CLR could also include using CLR in combination regimens together with more vigorous bactericidal drugs to clear MDR-TB. In this setting CLR may serve as a companion drug, increasing permeability to other antimicrobial agents and exerting its immunomodulatory effects. However, it should not be counted as or included among the drugs making up the standard regimen [4]. Furthermore, besides the observed *in vitro* synergistic effect of CLR and LZD [57], one clinical study also described an interaction between CLR and LZD suggesting an increase of LZD serum concentration when combined with CLR [61]. This could potentially be cost saving, because a reduced dose of the very expensive drug LZD can be used to reach similar drug exposure and this should be verified on a case by case basis by measuring LZD levels. The observed interaction also has a downside since the occurrence of major adverse events associated with LZD, such as anaemia and peripheral neuropathy, are higher when serum concentrations of LZD are higher. Therefore, this necessitates caution when prescribing CLR simultaneously with LZD [61].

Despite high MIC values obtained by MIC testing, bacteriostatic effects were seen with VCE and CFU counts *in vitro* [39, 45, 48, 49] and *in vivo* [39, 47, 59]. Furthermore, *in vivo* results showed a decrease in mortality when macrolides were added to the treatment regimen [39, 60]. These findings suggest CLR may serve as a lead compound for exploring the possibilities of structural modifications to create more potent derivatives. This strategy is already used for several other TB drugs. SQ109, the synthetic analogue of EMB, shows promising *in vitro* and *in vivo* anti-TB activity [79] and has completed three phase 1 studies. LZD, belonging to the oxazolidinone class of antimicrobials, has shown efficacy against MDR-TB [80, 81], but its associated serious adverse events have prompted development of new oxazolidinones, such as PNU-100480 [82] and AZD5847 [83]. Also in the case of metronidazole, a member of the nitroimidazole class of antimicrobials, the structurally related nitroimidazopyrans, PA824 and OP-67683, were found to possess potent activity against MDR-TB [84, 85].

In the large group of macrolides a new generation, known as the ketolides, has emerged. The ketolides include telithromycin, cethromycin and solithromycin. There are data suggesting that the mechanism of action of these derivatives is slightly different from earlier generations of macrolides. Ketolides appear to allow more proteins to pass through the ribosome during protein synthesis. By contrast, the macrolides lead to a complete or near-complete inhibition of protein synthesis. This partial inhibition of protein synthesis by ketolides is likely to cause more cellular deregulation, which appears to have a stronger bactericidal effect [86]. From these ketolides, fluoroketolides like K-1602, K-1636, K-1835 and K-1804 have been synthesised, which show improved MIC values against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* compared with erythromycin and the earlier generation ketolide, telithromycin [87]. This example shows the potential to improve antibacterial activity of analogues derived from a lead compound, which is initially classified as bacteriostatic. In addition, other fluoroketolides, like HMR-3562 and HMR-3787, have shown activity against several respiratory tract pathogens, like *S. aureus*, pneumococci and *Haemophilus* species [88]. However, for the ketolides discussed in this review it should be noted that the US Food and Drug Administration (FDA) strengthened their warning on the risk of liver toxicity when using telithromycin, the first FDA-approved ketolide [89]. The drugs should be used cautiously since *de novo* hepatotoxicity developed within a few days following treatment with telithromycin in three individuals with previously normal liver test results [90]. The authors judged the adverse drug reactions as probably but not definitely related to telithromycin. Moreover, the FDA strengthened warning regarding telithromycin-related adverse events including visual disturbances and loss of consciousness [89, 91].

Further limitations of macrolide treatment are on the grounds of adverse drug reactions and drug–drug interactions. In case reports macrolides are found to be associated with prolongation of the QT interval and Torsades de Pointes, prolonging the repolarisation period of the action potential by blocking the HERG potassium channels, similar to class III antiarrhythmics [92]. The European Society of Cardiology considers erythromycin and CLR as drugs that have the greatest potential for generating Torsades de Pointes and QT interval prolongation [93]. The proarrhythmic potential of the individual macrolide antibiotics is different as reported in a retrospective analysis of case reports that appeared in the FDA adverse event reporting system on macrolides and Torsade de Pointes. In 156 patients, proarrythmic potential was associated with erythromycin in 53% of cases and with CLR in 36% of cases, and 11%

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occurred in azalide-treated patients [93]. Since other MDR-TB drugs like moxifloxacin [94], delamanid [95] and bedaquiline [96] are also known to lengthen QT interval, adding macrolides to a MDR-TB regimen could increase the risk of cardiovascular events and mortality.

To summarise, although high MIC values are usually found for *M. tuberculosis* the potential benefits of macrolides are: their accumulation in relevant compartments and cells in the lungs; their immunomodulatory effects; and their ability to disorganise the cell envelope, leading to increased permeability to other bactericidal drugs. To conclude, macrolides deserve more research interest than they currently attract, as the current results, demonstrating synergistic effects, decreased mortality *in vivo* and inclusion in the treatment regimens of patients with MDR-TB, appear to be more promising than previously thought. The future for potential novel drugs for MDR-TB is brighter than ever with more drugs in the pipeline than over the past decades. Among these may be a more active macrocyclic drug with beneficial secondary effects to enhance the activity of the treatment regimen.

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