Biomedicine & Pharmacotherapy 105 (2018) 1353–1359

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Review

Clofazimine: A useful antibiotic for drug-resistant tuberculosis

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ARTICLE INFO

Keywords:
Mycobacterium tuberculosis
Clofazimine
Tuberculosis
Treatment

ABSTRACT

Drug resistance is still the major threat to global tuberculosis (TB) control, and drug-resistant (DR) *Mycobacterium tuberculosis* (*M. tuberculosis*) strains have become the main challenge worldwide. Currently used antibiotics for treatment of DR-TB are often poorly tolerated and not sufficiently effective. Since the therapeutic options are still limited, the main strategy for treatment of DR-TB is to repurpose existing anti-mycobacterial agents. Clofazimine (CFZ) is one such drug that has recently attracted interest against DR-TB. CFZ is a hydrophobic riminophenazine that was initially synthesized as an anti-TB antibiotic. Although the mechanisms of action of CFZ are not yet entirely understood, it has been suggested that outer membrane is its primary action site, and the respiratory chain and ion transporters are the putative targets. In this review, we will discuss the anti-mycobacterial properties of CFZ, and provide new insights into the clinical use of this drug.

1. Introduction

Tuberculosis (TB) has existed for millennia and remains the leading cause of infectious disease deaths globally, responsible for 1.7 million deaths in 2016 [1]. The current antibiotic treatment of active TB requires 6 months of combination therapy with the first-line drugs (FLDs) isoniazid (Inh), rifampicin (Rif), ethambutol (E) and pyrazinamide (Z). Inappropriate treatment, poor drug quality and inadequate drug intake or treatment response generates multidrug-resistant (MDR) strains (i.e. *Mycobacterium tuberculosis* bacilli resistant at least to Inh and Rif) and extensively drug-resistant (XDR) strains [i.e. MDR strains resistant to any fluoroquinolone and to at least one second-line injectable drug (SLID), amikacin (Am), capreomycin (Cm) or kanamycin (Km)]. Improper use of a second-line drug (SLD), for other infections, may contribute to generating XDR-TB. The global effort to end TB continues to face the threat of widespread dissemination of drug-resistant *M. tuberculosis* strains [2]. Current first-line anti-TB antibiotics are not sufficiently effective against DR-TB, thus, more toxic and less effective SLDs are necessary, with cure rates ranging from 36% to 50% [3]. Therefore, there is an urgent need for new drugs and approaches for the treatment of DR-TB. Since TB regimens are limited, a complementary approach is to repurpose existing antibiotics. Recently, novel therapeutic combinations for DR-TB involved the use of clofazimine (CFZ) [4]. The World Health Organization (WHO) listed this drug as a category C agent in the treatment of MDR- and XDR-TB [5].

CFZ was originally described in 1957 by Barry et al. [6] as a hydrophobic riminophenazine to be used specifically for the TB treatment, but monotherapy was unsuccessful in primates and humans, thus the drug was overlooked for decades [7,8]. The phenazine nucleus is the main structure of CFZ, with phenyl substituents and an R-imino group (Fig. 1). The R-imino group has key structural features for anti-mycobacterial activity, based on the halogens on the phenyl substituents at positions 3 and 10 of phenazine nucleus [9].

As CFZ was thought to be an ineffective anti-TB drug, in 1981 CFZ was recommended by WHO for treatment of leprosy in combination with Rif and dapsone [10]. The interest in CFZ-containing therapeutic regimen for TB has been recently revitalized after the study conducted by Van Deun et al. [11] showing that a regimen containing CFZ and other drugs including high-dose fluoroquinolones was very effective against MDR-TB and able to decrease the duration of therapy in

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https://doi.org/10.1016/j.biopha.2018.06.023
Received 16 April 2018; Received in revised form 3 June 2018; Accepted 4 June 2018

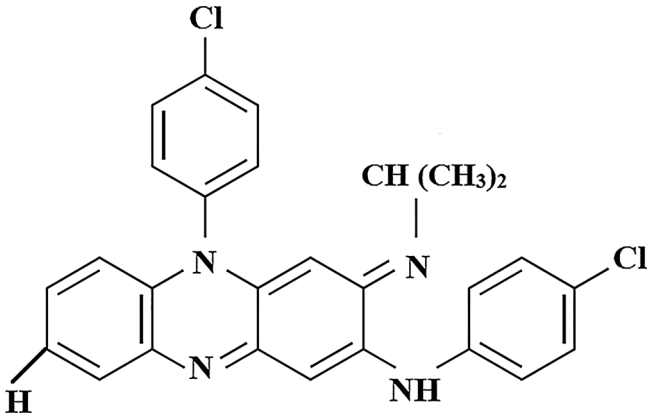

Fig. 1. Molecular structures of CFZ. [3-(p-chloroanilino-10- (p-chlorophenyl))-2, 10-dihydro-2-(isopropylimino)-phenazine]. *(The figure was modified from Igarashi et al. with permission from the publisher)* [69].

difficult-to-treat cases.

In the present study, we will review investigations elucidating the roles of CFZ in the control of DR-TB, discuss anti-mycobacterial properties of the drug, and provide new insights into the clinical use of treatment options.

2. Antimicrobial properties

2.1. Mechanisms of action

The mechanism(s) of antimicrobial action of CFZ is not entirely understood. However, it has been suggested that the primary action site of this antibiotic appears to be the outer membrane. The mycobacterial respiratory chain and ion transporters are the putative targets, and CFZ acts by inhibiting these targets [12]. Since the phenazine molecules are auto-oxidizable compounds, they could act as artificial electron acceptors. Therefore, respiratory system oxidizes CFZ instead of NADH, causing a reduction in the amount of ATP available for all cellular processes (Fig. 2) [13,14].

Because of highly lipophilicity and redox potential of CFZ, the anti-mycobacterial activity of this drug is based on oxidation of reduced CFZ, leading to the production of reactive oxygen species (Fig. 3). Some studies reported that CFZ selectively binds to guanine of DNA, therefore it is possible that this drug has a selective effect on DNA functions in *M. tuberculosis* [15]. In addition, CFZ can enhance the effect of bacterial phospholipase A2 and release lysophospholipids, the enzymatic hydrolysis products that are toxic to *M. tuberculosis*, leading to underpin the anti-mycobacterial effect of this drug [16].

Ammerman et al. have shown that CFZ has delayed anti-*M. tuberculosis* activity which was due to its mechanism of action. They have indicated that although in the first week of administration, CFZ did not demonstrate bactericidal activity at any concentration neither in vitro nor in vivo, it demonstrated concentration-dependent antimicrobial activity during the second week of exposure both in vitro and in vivo [17].

2.2. Spectrum of activity

CFZ has been used as an anti-leprosy drug to control erythema nodosum leprosum (in acute reactionary phases of leprosy), and to decrease the corticosteroid dose which is necessary to manage these patients [18]. With the advent of HIV, CFZ acquired new importance in the treatment of disseminated *Mycobacterium avium complex* (MAC) diseases [19]. CFZ has been successfully used, alone or in combination with other antibiotics, including clarithromycin, to control the bacteremia in HIV-MAC co-infected patients [20]. The drug is effective in

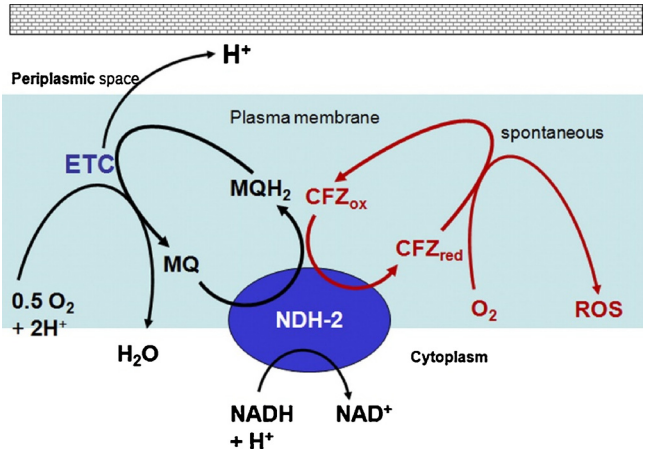

Fig. 2. CFZ is reduced by NADH dehydrogenase II to release reactive oxygen species upon reoxidation by O2 in *M. tuberculosis*. Depiction of CFZ-mediated redox cycling and ROS production. Diagram depicting menaquinone (MQ) of the respiratory chain and CFZ as competing substrates of NDH-2. The electron transport chain (ETC) in *M. tuberculosis* is primarily composed of two oxidoreductases in addition to NDH-2: cytochrome bc_1 complex, which is reduced by menaquinol, and cytochrome aa_3, which obtains electrons from cytochrome bc_1 and transfers them to O_2 in a coupled reaction that produces water and the translocation of protons from the cytoplasm to periplasmic space. Oxidation of reduced CFZ by oxygen occurs non enzymatically and produces ROS. *(The figure was modified from Yano et al. with permission from the publisher)* [12].

decreasing the mycobacterial counts and alleviating symptoms associated with MAC infection, in combination with Rif, E, and ciprofloxacin. CFZ can be used in non-tuberculous mycobacterial (NTM) infections (such as *M. marinum*, *M. hemophilum* and *M. kansasii* infections), in patients who are HIV positive and already co-infected with *M. ulcerans* [21].

CFZ can show sustained anti-mycobacterial activity in the mouse models of TB chemotherapy. In the mouse models that received CFZ, the regrowth of *M. tuberculosis* was delayed, compared to those that did not receive CFZ [22,23].

As to treatment of drug-resistant TB, two main reasons justifying the use of CFZ are i) efficient in vitro and in vivo activity against MDR and XDR strains [24,25] ii) very low rate of CFZ resistance among *M. tuberculosis* isolates [26,27].

2.3. Activity of clofazimine against M. tuberculosis biofilms and hypoxic cultures

Unlike the biofilms of other bacteria which consist of polysaccharides, lipids, proteins and DNA, the composition of mycobacterial biofilms contains an extracellular matrix rich in lipids rather than polysaccharides [28].

M. tuberculosis isolates residing in biofilms include slowly- or non-replicating (NR) (sessile) cells that are resistant to antibiotics and exponentially- or actively-replicating (AR) (planktonic), drug-susceptible (DS), cells [29].

Mothiba et al. [30] showed that CFZ can exhibit differential activities against AR, slowly replicating, and NR *M. tuberculosis*. In their study, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of CFZ against exponentially growing *M. tuberculosis* strains in planktonic cultures were 0.06 mg/L and 5 mg/L, respectively. In addition, the slowly replicating biofilm-producing isolates of *M. tuberculosis* were mostly susceptible to the bactericidal action of CFZ. CFZ exposure also resulted in dose-dependent inhibition of biofilm formation that achieved statistical significance at concentrations of 0.07 mg/L for *M. tuberculosis*. Overall, CFZ was active against planktonic and slowly replicating phases of *M.*

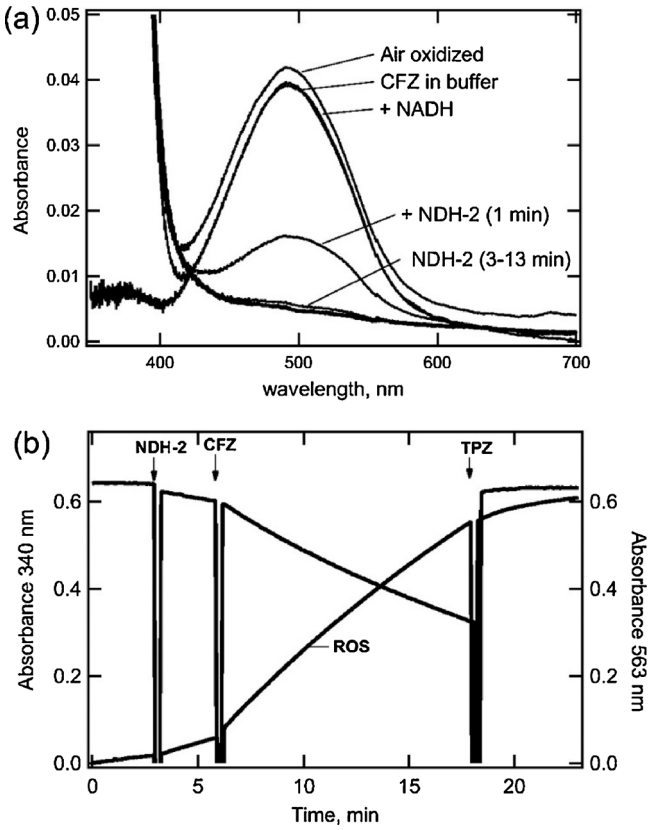

Fig. 3. Reduction of CFZ (a) and production of ROS (b) catalyzed by M. tuberculosis NDH-2a). It shows CFZ reduction by NDH-2 under anaerobic conditions and subsequent reoxidation. Stock CFZ in Me_2SO is added to a solution of 50 mM MOPS/Na^+, pH 6.5, 2.0 mM $MgCl_2$. Approximately 70% of the CFZ remained in solution exhibiting a spectrum consistent with cationic CFZ. NADH is then added; it did not produce a significant change during a 10-min incubation period (+ NADH). Purified NDH-2 is then added to start the reaction, and additional spectra are taken at the indicated times. After the absorbance loss is complete, cuvette is exposed to air after which a spectrum is recorded. **b)** It shows ROS production by purified NDH-2. NADH oxidation and ROS generation, catalyzed by NDH-2 is measured under aerobic conditions. Reactions are performed in a total volume of 1.0 ml and contained 200 μM NADH with an ROS detection system. At the indicated times, NDH-2 is added to final concentration of 2.0 μg/ml, followed by CFZ or TPZ. *(The figure was* adopted *and* reproduced *from Yano et al.* with permission from the *publisher)* [12].

tuberculosis but was ineffective against NR, biofilm-encased phases (Figs. 4 and 5) [30].

Non-replicating *M. tuberculosis* is also present in the tissues of two billion people in the world with latent TB infection (LTBI), with a 10% lifetime risk of reactivation to TB disease [31]. Identification of drug targets in NR dormant persisters (genotypically susceptible but phenotypically drug-tolerant, drug-tolerant cells) living in caseum of human tuberculosis granulomas is critically important to achieving the goal of complete TB eradication [32]. Several in vitro models have been developed to obtain NR *M. tuberculosis*. One of the most popular of them is the Wayne dormancy model of hypoxia [31]. In this model, the dormant *M. tuberculosis* is obtained by gradual adaptation of stirred cultures of aerobic *M. tuberculosis* to anaerobiosis through the self-generated formation of an oxygen gradient. Iacobino et al [33] showed that CFZ is active in hypoxia at pH 5.8 but not at pH 7.3 (mimicking environments of cellular and caseous granulomas, respectively), in keeping with the knowledge of low penetration and extreme drug tolerance of *M. tuberculosis* to several drugs including CFZ in caseum, with exception of rifamycins [34,35].

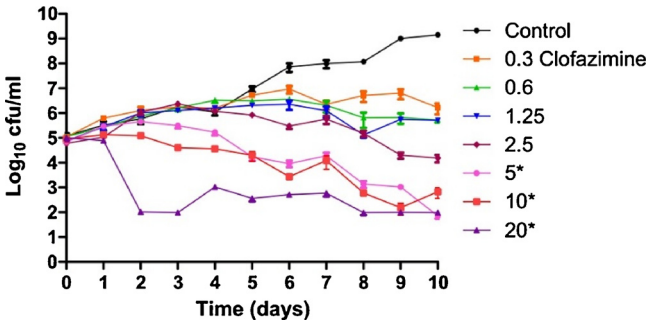

Fig. 4. Clofazimine (0.3–20 mg/L) minimum bactericidal concentrations (MBCs) and time–kill curves for planktonic bacilli of *Mycobacterium tuberculosis*. The results are for three separate experiments performed in duplicate for each concentration of clofazimine and are presented as the mean ± standard error of the mean. The number of bacteria on Day 0 was 1.2 × 105 ± 2.9 × 104 CFU/mL and the maximum growth achieved in the control on Day 10 was 1.4 × 109 ± 1.6 108 CFU/mL. The MBC was achieved at 5 mg/L. The rate of bacterial killing for each concentration of the antibiotic was determined daily for 10 days. *(The figure was adopted and reproduced from Mothiba* et al. *with permission from the publisher)* [30].

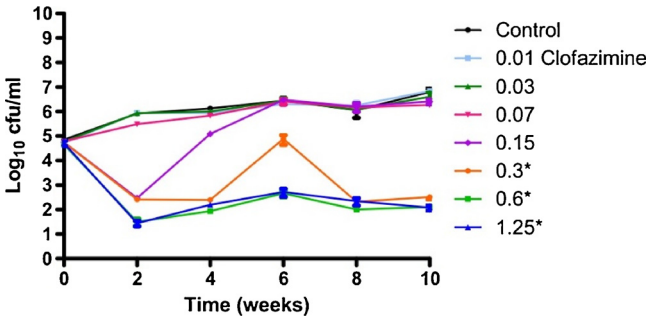

Fig. 5. Clofazimine (0.01–1.25 mg/L) minimum bactericidal concentrations (MBCs) and time–kill curves for biofilm-producing bacilli of *Mycobacterium tuberculosis*. The results are for three separate experiments performed in duplicate for each concentration of clofazimine and are presented as the mean ± standard error of the mean. The number of bacteria at Week 0 was 1.3 × 10^5 ± 3 10^3 CFU/mL and the maximum growth achieved in the control at Week 10 was 6.2 × 10^6 ± 2.6 × 10^6 CFU/mL. The MBC was achieved at 0.3 mg/L. The rate of bacterial killing for each concentration of the antibiotic was determined biweekly for 10 weeks. *(The figure was adopted and reproduced from Mothiba* et al. *with permission from the publisher)* [30].

3. Synergism

3.1. Synergism between clofazimine and common TB drugs

TB chemotherapy needs a treatment regimen containing a combination of antimycobacterial drugs for several months; therefore, it is mandatory to study the synergism of drugs in preclinical studies. Several investigations have been carried out on the activities of CFZ when combined with commonly used first and second-line anti-TB drugs [8].

In a study by Zhang Z. et al. [36], the synergistic activities between CFZ and E, Am, Cm, levofloxacin (Lfx) and moxifloxacin (Mfx), were evaluated against MDR-TB isolates. In this study, the fractional inhibitory concentration (FICs) indexes showed the synergy in approximately 46%, 25%, 33%, 16% and 21% of isolates in combinations containing CFZ and E, or Lfx, Mfx, Am, and Cm, respectively.

Lopez-Gavin et al. [37] compared the efficacy of three combinations including CFZ + pretomanid (Pto) + Lfx, CFZ + Pto + Mfx and CFZ + Pto + UB8902 against MDR and DS *M. tuberculosis* strains. They showed that the FICs of all combinations ranged from 1.2 to 2.3, which suggests an additive interaction against all the isolates.

In another study, Zhang S. et al. [38] evaluated the effects of CFZ in combination with Rif, E, Z, Am, Mfx, Lfx or para-amino salicylicacid (Pas). They found that Z was by far the most active antibiotic in increasing the CFZ activity, followed by Rif, Mfx, Lfx, Am, and Pas in decreasing order of activity. In this study, E had no apparent effect on increasing the CFZ activity.

Lu et al. [39] explored the role of CFZ in combination with anti-TB drugs. The FICs of CFZ in combination with PAS, Cm, Pto, and clarithromycin were < 0.5, *i.e* a synergistic effect. In addition, all antibiotics reduced the numbers of colony forming units in the lungs and spleens of *M. tuberculosis*-infected mice, compared with the controls.

3.2. Synergism between clofazimine and benzothiazinones

Benzothiazinone (BTZ) is an extremely potent class of antibiotics active against both DS and DR *M. tuberculosis*. The potency of these drugs has been defined by their specificity for critical cysteine 387 residues of the decaprenyl-phosphoryl-D-ribose oxidase encoded by the gene *dprE1* [40].

BTZ in combination with anti-TB drugs is able to target dormant *M. tuberculosis* isolates and inhibit the ATP synthase, an enzyme that can maintain bacterial viability during dormancy. Furthermore, the residual metabolism of dormant *M. tuberculosis* needs de novo RNA and protein synthesis for survival during the reactivation. Given the sensitivity of NR *M. tuberculosis* to inhibit these processes, combining BTZ and other drugs active such us CFZ against NR cells may be important for new TB regimens [41].

The 2-piperazino-benzothiazinone 169 (PBTZ169) is a piperazinobenzothiazinone derivative which has several advantages compared to other benzothiazinones (easier chemical synthesis, lower cost of goods and better pharmacodynamics) [42]. Lechartier et al. [43] showed that CFZ has a synergistic effect in combination with PBTZ169 against NR *M. tuberculosis*. The synergy between CFZ and PBTZ169 was lost in menaquinone (MK-4)-rich medium, showing that this electron transfer chain cofactor is the probable link between their activities. The CFZ-PBTZ169 combination was also tested in vivo, where a great reduction in mycobacterial load was obtained in a chronic TB murine model. These data confirm the synergic activity of CFZ together with PBTZ169 as a promising combination against DR *M. tuberculosis*. However, due to these experimental drugs (BTZ) have not yet been tested in humans, they may not be used in humans depending on the unknown in vivo activity and need more experimental tests in human volunteers.

3.3. Synergism between clofazimine and MmpL3 inhibitors

MmpL3 proteins are transporters from the Mycobacterial Membrane Protein Large (MmpL) family that belong to the resistance-nodulation-cell division (RND) superfamily transporter. The membrane proteins MmpL3, have been implicated in the formation of *M. tuberculosis* outer membrane and are required for the export of trehalose monomycolate form of mycolic acids to the outer membrane or periplasmic space of *M. tuberculosis*. The anti-TB drug candidate SQ109 is an ethylenediamine analog of E which has been identified by whole-cell screening. SQ109 was shown to abolish the MmpL3-mediated trehalose monomycolates export [44].

The SQ109 + CFZ combination (MmpL3 inhibitors) showed synergistic activity both in vitro and *ex vivo*. Therefore, using the checkerboard method is needed to study the interaction profiles of anti-TB drugs with two different antibiotics inhibiting MmpL3 [45].

Li et al. [46] showed that MmpL3 inhibitors act synergistically with CFZ. Four inhibitors were tested including indolamide NITD-304, indolamide NITD-349, adamantyl ureas AU1235, and adamantyl urease AU36. All inhibitors enhanced *M. tuberculosis* susceptibility to CFZ, likely due to increased penetration of compounds caused by alterations in outer membrane assembling, and synergistic effect of inhibitors with CFZ, affecting energy metabolism [46].

4. Pharmacokinetics and pharmacodynamics

CFZ is present in both free and bound states in plasma. This antibiotic can be found attached to a carrier which transports it across the membrane of target cells and also may be free and accumulate by an active transport mechanism [47].

Nix et al. showed the pharmacokinetics and bioavailability of CFZ in the presence of food, orange juice, and antacid. CFZ indicated a variable prolonged lag-time with a high mean apparent volume of distribution (1470 L(Liters)) and high mean oral clearance (76 L/h (Liters/hour)). The bioavailability was associated with several individual variabilities which the high-fat meal was the main factor. The antacid and orange juice decreased the mean bioavailability of CFZ [48].

In another study, a two-compartment pharmacodynamics model was recommended according to experimental models. Compartment I was evident with short-term low dosages and had an elimination half-life of approximately 7 days while compartment II showed a second elimination half-life of 70 days. However, slow elimination did not seem to be related to a drug-crystals formation in the body. Three CFZ metabolites were identified in the urine. The first was generated by hydrolytic dehalogenation, the second by hydrolytic deamination followed by glucuronidation of a hydroxyl group, the third by hydration followed by a glucuronidation process [49].

McDougall evaluated pharmacokinetics and pharmacodynamics of CFZ in adults with leprosy. He showed that because of aqueous insolubility of CFZ, absorption after intramuscular injection is very slow. Therefore, the oral route is the only way to administer CFZ. Due to very slow excretion in the urine, very little CFZ appears to reach the excretory portions of the kidney, showing that little or none of the plasma CFZ is in free solution. Furthermore, CFZ bound appreciably to serum lipoproteins, not globulin and albumin [50].

CFZ has two major target areas including the cells of the reticuloendothelial system and adipose tissues. Hence, any intracellular drug influence can be related to plasma levels. In addition, CFZ crystals are found in bone, muscle, skin, heart, eye, gallbladder, nervous tissue, where they remain for years after cessation of administration. CFZ also can cross the blood-brain barrier in very small amounts. In *M. tuberculosis*-infected mice the levels of antibiotic in the brain are low while being high in spleen, liver, lung, adipose tissue, and mesentery [47].

5. Clinical treatment for TB

5.1. Use of CFZ in patients with DR-TB

Administration of CFZ to drug-resistant patients was associated with adverse events, the most common of which was discoloration of the skin and mucous membranes [7]. Due to the lack of CFZ standardized regimens for treatment of drug-resistant TB, different daily dosages were evaluated, ranging from 50 to 100 mg [4,51], with few reaching 300 mg [7]. The CFZ dosage for treatment of multibacillary leprosy is 50 mg/day plus 300 mg given once a month under supervision [52]. CFZ and bedaquiline therapy were reported to be associated with an increased QT interval and cardiac arrhythmia; WHO guidelines recommend to perform weekly electrocardiograms during the first months of therapy [7,53].

Several studies indicated that CFZ has good activity against MDR strains both in vitro and in animal model studies. An important feature of CFZ was that activity in mice was comparable to that of Inh and Rif [51,54] and showed good efficacy and low toxicity against MDR and XDR strains [55].

Treatment of MDR-TB needs the prolonged use of SLDs, which are more toxic and less efficacious than FLD. In a randomized controlled study carried out by Tang et al. [55] performed in China, 105 MDR-

patients were randomly assigned to the CFZ therapy and control groups. Sputum culture conversion to negativity and cavity closure occurred earlier in CFZ therapy than in the control group. In addition, treatment success rate in patients who received CFZ was higher (approximately 75%) than in controls. In this study use of CFZ was associated with faster cavity closure, accelerated sputum culture conversion, and improved treatment success rates.

Van Deun et al [11] performed a study on SLDs-untreated MDR-TB patients and reported treatment outcome of all patients. CFZ was one of the most effective antibiotics in combination therapy and serial regimen formulations resulted in treatment outcomes comparable to those obtained with FLD.

In a retrospective cohort study done by Padayatchi et al. [56] in South Africa on 85 XDR-patients of which 50 patients were treated with CFZ-containing combinations and 35 patients with non-CFZ-containing, the CFZ-containing regimen had a higher rate of culture conversion (40%), with 2-fold increase at 6 months. In addition, CFZ was associated with improved culture conversion among the XDR-TB/HIV co-infected patients. Adverse effects were minor and non-life-threatening [56].

Also, Chu et al. [57] performed a randomized clinical trial and evaluated the treatment regimen of 189 patients infected with *M. tuberculosis* isolates from 18 hospitals (140 MDR-TB cases and 49 XDR-TB). Results showed that cure rates in regimens including or excluding CFZ were 67% and 48.5%, respectively, hence, regimens including CFZ had a better effect. The majority of patients tolerated CFZ, and long-term use had good safety.

In another study carried out by Dalcolmo et al. [58] in Brazil on effectiveness and safety of CFZ in MDR-TB, they indicate that the drug is effective at the programmatic level, safe and, does not increase the frequency of CFZ resistance. This study showed that CFZ had the potential to further contribute to the successful treatment of MDR-TB cases.

5.2. Use of CFZ in TB-leprosy co-infections

Despite progress in treatment and living conditions, there are more than 10 million new TB cases and more than 200,000 new leprosy cases per year globally [59]. Therefore, there is a requirement for screening of patients with *M. tuberculosis* in patients diagnosed with leprosy, to prevent generation of DR-TB.

Trindade et al [59]. reported two cases of patients co-infected with *M. tuberculosis and Mycobacterium leprae*. Both had type 1 leprosy reactions; the first had pleural TB and the second pulmonary TB. Both patients were successfully treated with CFZ.

In another study performed by Sendrasoa et al. [52], it was reported a case of pulmonary TB diagnosed in a patient treated with glucocorticoids for type II leprosy. The patient was cured by using the standard therapy including dapsone- Rif-CFZ; after twelve months, skin lesions disappeared, the slit skin smear test was negative, and the patient recovered completely.

In the study of Al-Mendalawi [60], it was described a case of MDR-TB with multibacillary leprosy treated with dapsone- Rif-CFZ. In this case, the interplay between the two infections was prescribed by daily use of CFZ. Finally, at the end of therapy, sputum positive cultures were negative and the patient was recovered.

Verma et al. [61] reported the case of a patient with *M. tuberculosis* sputum positive complicated by a type-II leprosy reaction, based on clinical findings. Also, this patient was successfully treated with dapsone- Rif-CFZ. Finally, the patient described by Ganesan et al. [62], with oral TB of the tongue and lepromatous leprosy was successfully treated with thalidomide plus CFZ.

According to these studies, CFZ can be used alone or combined with other anti-mycobacterial drugs for leprosy patients who co-infected with MDR- or XDR-TB.

5.3. Use for patients who have nontuberculous mycobacteria

Nontuberculous mycobacteria are increasing especially in areas in which TB is decreasing. The combination CFZ + Am had synergistic activity against rapidly growing NTM species *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobacterium fortuitum*, MAC, and *Mycobacterium simiae*. In two studies, CFZ was used in the place of Rif in lung disease caused by MAC, with similar outcomes [63,64].

In the study of Singh et al. [65], it was shown that CFZ was the most effective drug against *M. abscessus* complex, and that the combination CFZ + tigecycline had a promising synergistic effect.

Ferro et al. [66] investigated the interactions between CFZ and Am or clarithromycin (CL), and the contribution of these drugs in the treatment of *M. abscessus* and MAC infections. CFZ alone was bacteriostatic for *M. abscessus* and MAC, but CFZ + Am was synergistic against both NTM species. In addition, CFZ + CL was also synergistic against *M. abscessus* and MAC

In another study [67], the outcomes of patients treated with CFZ and Rif were evaluated. The majority of patients were treated with CFZ + macrolide or CFZ + E, and 95% of them converted from positive to negative sputum cultures. Moreover, a significant proportion of CFZ-treated patients became negative compared with those treated with Rif (100% vs 71%). These studies showed that CFZ may be considered as an alternative drug for treatment of MAC lung disease.

Shen et al. [68] demonstrated that CFZ was highly efficacious for treatment of infections caused by rapidly growing mycobacteria (RGM), including *M. abscessus*, *M. fortuitum*, *M. chelonae*. Approximately 95% of *M. abscessus*, *M. fortuitum* and *M. chelonae* isolates had CFZ MICs of ≤ 1 mg/L. The MIC_{50} of CFZ ranged from 0.25 to 0.5 mg/L and the MIC_{90} from 0.5 to 1.0 mg/L. Therefore, CFZ can be a promising drug for treatment of RGM infections.

6. Conclusion

The increasing incidence of MDR- and XDR-TB worldwide raise the urgent need for more effective drugs and/or therapeutic regimens, especially in TB + HIV co-infected patients with high mortality rates. Since therapeutic regimens for DR-TB are still limited, a complementary approach is to repurpose existing antibiotics. The available evidence recommends CFZ-containing as an additional promising treatment option for DR-TB, although the duration of use and optimal dosages of CFZ need further investigations.

Transparency declarations

None to declare.

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