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

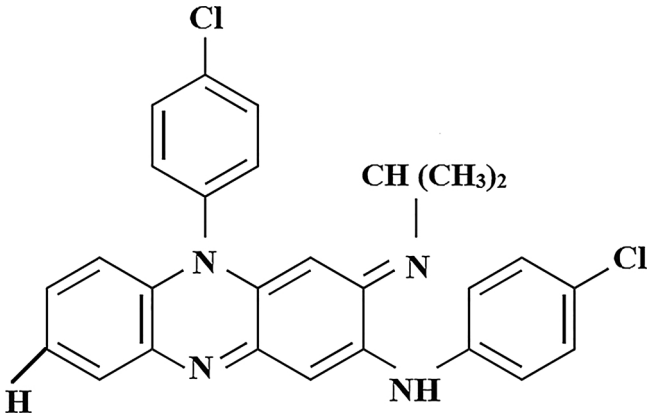

Fig. 1. Molecular structures of CFZ. [3-(p-chloroanilino-10- (p-chlorophenyl))-2, 10-dihydro-2-(isopropylimino)-phenazine]. *(The figure was modified from Igarashi et al. with permission from the publisher)* [69].

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

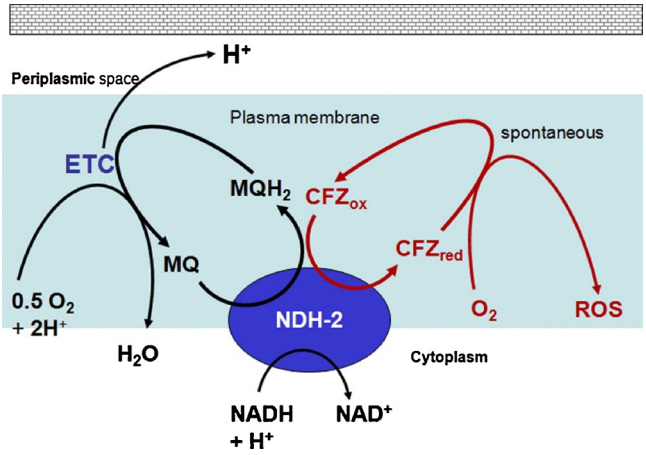

Fig. 2. CFZ is reduced by NADH dehydrogenase II to release reactive oxygen species upon reoxidation by O2 in *M. tuberculosis*. Depiction of CFZ-mediated redox cycling and ROS production. Diagram depicting menaquinone (MQ) of the respiratory chain and CFZ as competing substrates of NDH-2. The electron 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.*