Rifampicin and Bedaquiline: New Insights into Treating Tuberculosis

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Keywords: Tuberculosis, Drug Mechanism, Drug Resistance, Modes of Delivery, Drug Economics.

Abstract: Tuberculosis (TB), the Mycobacterium tuberculosis infection, remains as a severe issue around the globe, killing 1.4 million people in 2019. In this review, rifampicin, a major component of multidrug regimen, and bedaquiline, a novel drug specifically treats multidrug-resistant TB, are discussed and compared. Rifampicin (Rif) inhibits RNA polymerase by binding to the β subunit and blocks the elongation of transcription, while bedaquiline inhibits the F-ATP synthase by preventing c-ring rotation when it binds to the c subunit. However, multidrug-resistant TB (MDR-TB) has become severe. Mutations can result in key amino acid substitutions in conformational changes of RNA polymerase, disabling rifampicin to bind. New techniques and possibilities in the mode of delivery are also explored, as oral rifampicin can be improved by solid self-nanoemulsifying drug delivery system (S-SNEDDS) and bedaquiline can be improved by inhalation and long-acting injection.

1 INTRODUCTION

1.1 Overview of Tuberculosis (TB)

Tuberculosis (TB) is a bacterial infection caused by *Mycobacterium tuberculosis.* It can be spread from person to person via air. TB usually occurs on the lungs, but it can take place in kidneys, spine, or brain as well.

There are two types of TB, latent and active. People infected by latent TB would not have any symptoms and would not spread the disease. Latent TB can only be detected by tuberculin or TB blood test. While some people can develop active TB from latent TB the bacteria overcome the immune system and begin to reproduce. *Mycobacterium tuberculosis* can multiply and damage human body issues. Active TB patients are possibly spread the bacteria. People with deficient immune system, such as HIV carriers, are likely to get active TB.

1.2 Mortailities

Up to 2019, the estimated number of people with latent tuberculosis infection (LTIB) is one-quarter of the world's population, who are potentially infectors of reactivated TB (Cohen, Adam, 2019). In 2019, According to WHO, 1.2 million children caught TB due to the difficulty of diagnosing, and 1.4 million people died in 2019 due to TB. A person has 5-10% lifetime risk to get active TB when he or she is infected with bacteria *Mycobacterium tuberculosis*, and 45% of active TB infectors (without HIV) would die.

1.3 Typical Symptoms

Active TB can lead to coughing with or without blood and mucus, chest pain, loss of weight, fever, fatigue, night sweats, loss of appetite, etc. (Centers for Disease Control and Prevention, 2016). Recently, depression and anxiety were found to be common among pulmonary tuberculosis (PTB) infectors (Wang, 2018). Usually, the symptoms are mild for several months, which result in transmissions to other people.

1.4 Significance in China and the World

In China, the incidence of TB has reduced 24% from 2010 to 2019, but China still remains as a high-burden TB country with 833,000 TB patients in 2019 (WHO China TB treatment).

In 2019, most new TB cases took place in South-East Asian region (44%), African region (25%), and Western Pacific (18%) (World Health Organization, 2020).

Gu, M.
Rifampicin and Bedaquiline: New Insights into Treating Tuberculosis.
DOI: 10.5220/0011382200003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 1179-1187
ISBN: 978-989-758-595-1

1.5 Drugs for Tuberculosis and Their History

Isoniazid, developed in 1952, is considered as a highly critical for human medicine by WHO (World Health Organization, 2015). It was first synthesized by Meyer and Malley in 2019, while its anti-tuberculosis activity was discovered in 1945, being part of the combined drug regimen to solve streptomycin resistance (Fernandes, 2017). Also in 1952, pyrazinamide was found out to be effective at tuberculosis (Zhang, 2014). Then, ethambutol was introduced in 1961. In 1966, Rifampicin was developed by the Lepetit group as a semisynthetic drug from *Amycolatopsis rifamycinica* (Sensi, 1983). These four drugs consist the combination therapy.

As multidrug-resistant tuberculosis (MDR-TB) appears, second-line drugs such as cycloserine, ethionamide, and bedaquiline are applied.

Rifampicin, a widely used first-line drug for TB, and bedaquiline, the latest drug developed in 2012 for treating MDR-TB, are evaluated in this literature review to discuss the futural development of anti-TB drugs.

2 DRUG PHARMOCOLOGY

2.1 Introduction of Rifampicin and Bedaquiline

Rifampicin is one of the frequently prescribed first-line drugs for treating TB, while bedaquiline is a relatively novel drug to treat multidrug-resistant Tuberculosis (TB), especially patients who are resistance to rifampicin (Centers for Disease Control and Prevention, 2016); (PubChem).

This literature review covers from rifampicin and bedaquiline chemical structure, drug mechanisms, new insights into the modes of delivery, and drug economics. This include details of how rifampicin inhibits bacterial RNA polymerase by blocking transcription elongation and how bedaquiline inhibits F-ATP synthase by halting c-ring rotation are included. This review also discusses how oral rifampicin is improved by self-nanoemulsifying drug delivery system (SNEDDS) and how bedaquiline can be possibly delivered by inhalation and injection.

This review aims to compare rifampicin, a conventional anti-TB drug, and bedaquiline, a novel drug, evaluating the futural uses in TB treatment.

2.2 Chemical Structure

Rifampicin is a semi-synthetic drug derived from rifamycin B (a macrolactam antibiotic), belonging to ansamycins, as it contains an aliphatic ansa chain (PubChem). Rifampicin is also a polyketide with alternating carbonyl groups and methylene groups.

To synthesize rifampicin, aqueous solution of rifampicin is oxided to rifampicin S. Then, the rifampicin S quinone structure is reduced with hydrogen, giving off rifampicin SV. The rifampicin SV undergoes aminomethylation and turns into 3-pyrrolidi nomethylrifampicin SV. The product is oxidized to an enamine and is then hydrolyzed to 3-formylrifamicin SV, which reacts with 1-amino-4-methylpiperazine to give off rifampicin.

Bedaquiline, or 1-(6-bromo-2-methoxy-quinolin-3-yl)-4-dimethylamino-2-naphthalen-1-yl-1-phenyl-butan-2-ol, is a compound in the diarylquinoline group (Andries, 2005). Bedaquiline contains a quinolinic central heterocyclic nucleus with alcohol and amine side chains, which are responsible for the antimycobacterial property (Pontali, Emanuele, 2016).

2.3 Mechanism

2.3.1 Rifampicin (Rif) Is A N-Amino-N′-Methylpiperazine Derivative from Rifamycin

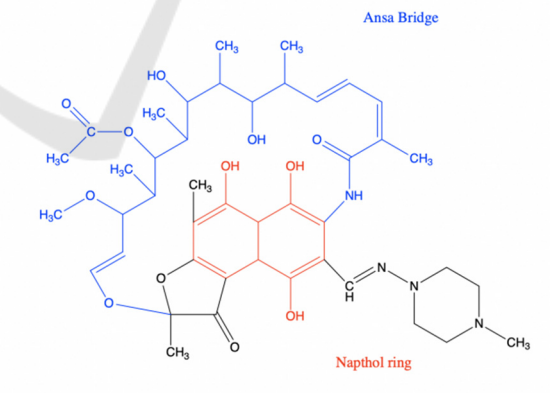

Figure 1: The chemical structure of rifampicin.

As shown in Figure 1, the blue part indicates the Ansa bridge and the red part indicates the Napthol ring. The graph depicts a 2-D chemical structure of the drug.

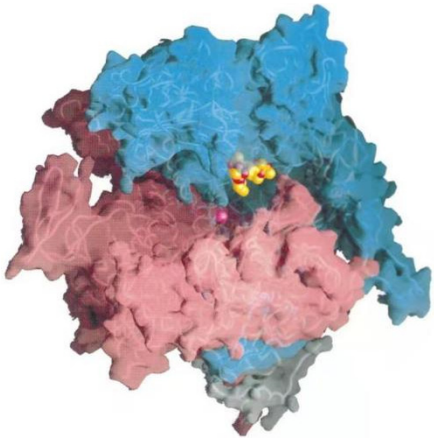

Figure 2: Rif-RNAP cocrystal structure (Campbell, Elizabeth A, 2001).

As shown in Figure 2, RNAP backbone is presented by tubes, with transparent molecular surface. Cyan: β; Pink: β'; white: ω. Rifampicin shown as CPK atoms, in which carbon is orange, oxygen is red, nitrogen is blue. The magenta sphere stands for Mg^{2+} ion at the active site.

Rifampicin can inhibit the bacterial RNA polymerase (RNAP). RNAP, an enzyme catalyzes the synthesis of mRNA from a DNA template, can no longer produce mRNA and thus the bacteria would be unable to produce essential proteins. This inhibition makes rifampicin a bactericidal drug. The inhibitor binds to the pocket of RNAP β sub-unit in the DNA channel or RNA channel (Campbell, Elizabeth A, 2001)

Rifampicin contains two polar groups on napthol ring and three polar groups on the ansa bridge (Figure 1), which five form hydrogen bonding with the binding pocket, and van der Waals association is formed by hydrophobic side chains around the napthol ring of rifampicin. Rifampicin thus blocks the path of RNA elongation when the transcript is 2 or 3 nucleotides long, depending on the phosphate group of the initiating nucleotide.

Rifampicin approximately doubles the apparent Michaelis constant of initiating substrate at the RNAP's i-site, while not affecting the second nucleotide binding to i+1 site (McClure, 1978). The formation of the first phosphodiester bond between these two nucleotides is catalyzed by RNAP. RNAP would translocate the 2nt transcript upstream, causing i+1 site replaces i-site (-1 position), and i-site nucleotide shifts to -2 position, if the initiating nucleotide contained a 5' triphosphate (Campbell, Elizabeth A, 2001). When the nucleotide is shifted to -2 position, it would clash sterically with rifampicin. Therefore, the RNAP is stuck at the position, and repeatedly produce 2nt transcript every time. However, if the initiating nucleotide carries a 5' diphosphate or monophosphate, then the formation of the first phosphodiester bond would be normal, but the second phosphodiester bond would fail due to the steric clash with rifampicin, resulting in 3nt transcript being repeatedly produced.

To sum up, rifampicin is bactericidal because it sterically blocks the elongation process of the transcription, as it binds to the β sub-unit in DNA or RNA channel of the RNAP.

2.3.2 Bedaquiline (BDQ)

BDQ is a diarylquinoline that treats MDR-TB.

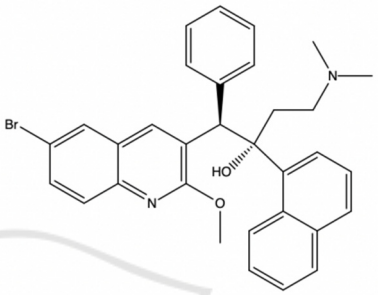

Figure 3: The chemical structure of Bedaquiline.

As shown in Figure 3, bedaquiline is displayed by a 2-D structure. Each solid line represents intramolecular bonding, with ends representing Carbon. Other elements are shown by letters.

Generally, Bedaquiline inhibits the F-ATP synthase of the *Mycobacterium tuberculosis* to stop ATP production. This is done by three mechanisms of the BDQ.

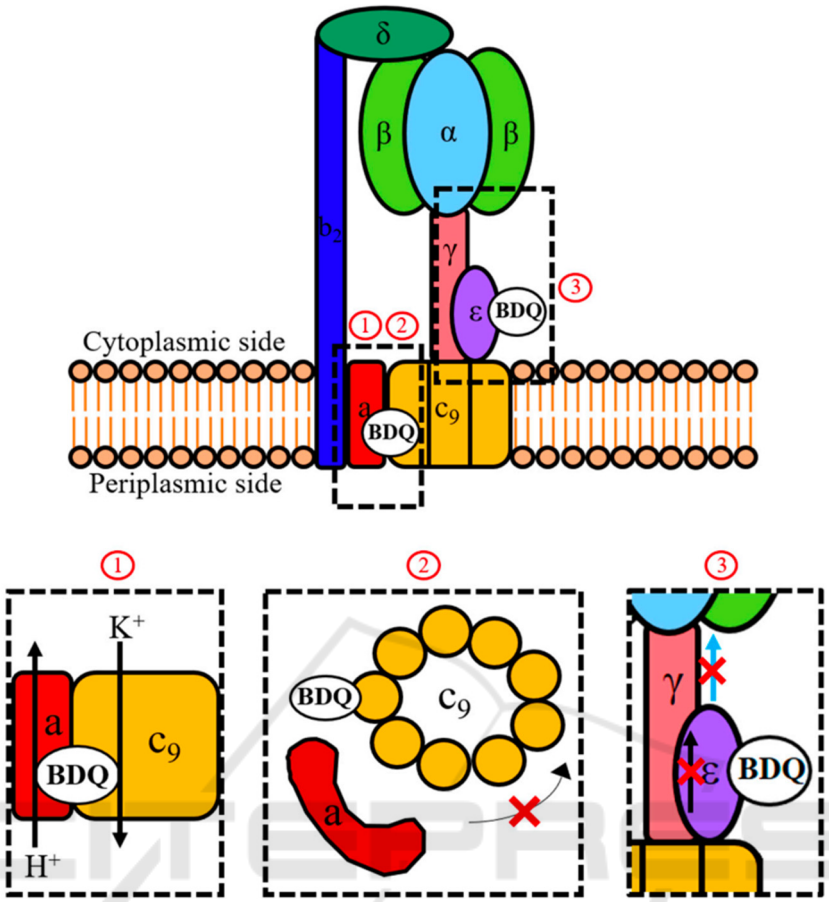

Figure 4: General mechanism of bedaquiline (Sarathy, 2019).

As shown in Figure 4, BDQ functions at the cell membrane and inhibits ATP synthase. No. 1 visualizes BDQ blocking the flow of proton, no. 2 visualizes BDQ stopping c-ring rotation by binding to ε-subunit, and no. 3 shows BDQ uncoupling electron transportation.

First, BDQ can halt c-ring rotation by binding to the c-ring on mycobacterial F-ATP synthase (Preiss, 2015). The c-ring has two c-submits, which have a cleft between them. The cleft is the binding site of BDQ, consisting of *M. phlei* counterparts of D28, E61, A63, I66 amino acid residues (Preiss, 2015). The c-ring can be bound by several BDQ molecules at the same time, and the binding affinity of a single BDQ molecule would increase if another BDQ molecule is joined to form complementary binding (Salifu, 2019). The binding to the c-ring would be sterically blocked and unable to pass between the interface of the F-ATP synthase's a-subunit and c-ring. The interaction between BDQ and E61 amino acid residue would prevent the ion exchange, an essential process for the flow of proton down the transmembrane pH gradient. Therefore, F-ATP synthase activity is halted and ATP synthesis is prevented.

The second target of BDQ is the ε-subunit, is also related with the c-ring rotation, on the F-ATP synthase (Biuković, 2012). Mtb ε-subunit connects c-ring rotation to the ATP formation at the $\alpha_3\beta_3$-headpiece, and it has an interdomain amino acid interaction network that can deliver information on c-ring rotation to its C-terminus (Sarathy, 2019). The C-terminus leads to conformation to contact with $\alpha_3\beta_3$-headpiece and transmits the information. However, BDQ binds to the Mtb ε-subunit at A10-W16 amino acid region (Biuković, 2012) and leads to intra-protein structural changes, damaging the intra-subunit communication network and thus inhibiting the Mtb ε-subunit function of connecting c-ring rotation to the ATP synthesis. The target's effect is currently considered as secondary, because, first, BDQ only has moderate binding to the ε-subunit (Biuković, 2012), and second, the BDQ resistance mutations in Mtb only occurs to the c-subunit (Segala, 2012).

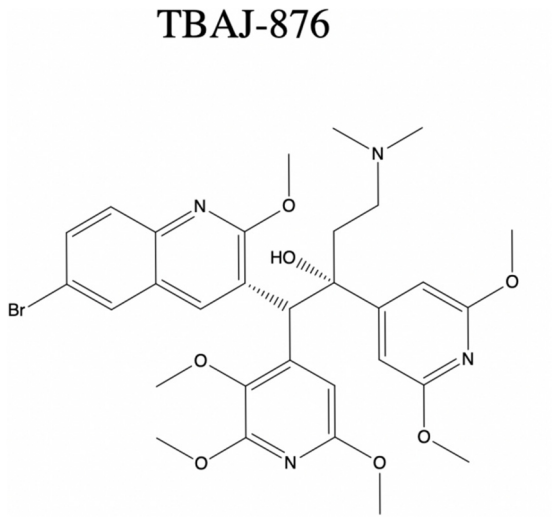

Figure 5: Chemical structure of analogue of bedaquiline, TBAJ-876.

As shown in Figure 5, structure is analyzed by 2-D chemical structure, which can be compared with bedaquiline structure in Figure 4.

The third mechanism, uncoupling electron transport from ATP synthesis, is still left controversial. TBAJ-876, a new BDQ analogue included in Sarathy et al. research, achieves a similar antibacterial property of BDQ. The TBAJ-876 has the same two mechanisms covered previously, but it doesn't have BDQ's uncoupler activity (Sarathy, 2019). This indicates that uncoupler activity is not an essential part of anti-mycobacterial activity.

3 DRUG-RESISTANT TUBERCULOSIS

3.1 Significance

The challenge of controlling tuberculosis is now even furthered due to multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) (Dheda, 2017). Drug-resistant TB is responsible for approximately 25% of global TB mortality and is trickly to cure (World Health Organization, 2020). Furthermore, the cost of treating MDR-TB is significant, as 54% of the fund ($2.26 billion out of $4.2 billion) was spent on the treatment and diagnosis of MDR-TB in 2020 (World Health Organization, 2020).

3.2 Rifampicin Resistance

Rifampicin, a major component of multidrug regimens to treat TB, is found to be ineffective at MDR-TB. The rifampicin resistance is caused by the conformational changes of RNA polymerase, as substitution of the key amino acids can alter the structure of RNA polymerase. These changes of amino acids in β subunit of RNAP would prevent the binding of rifampicin to the enzyme and thus develop rifampicin resistance (Telenti, 1993).

3.3 Bedaquiline Resistance

Even bedaquiline, the novel drug specific for treating MDR-TB and XDR-TB, has been reported with resistance. Due to incomplete treatment, mutations in the *atp*E gene can stop BDQ from binding to the c-subunit of the F-ATP synthase (Koul, 2007). In this way, BDQ cannot inhibit ATP production and its bactericidal effect diminishes.

Overall, drug-resistant TB circumstances should be strictly supervised. Not only conventional regimen should be improved by optimization, but also novel drug such as bedaquiline should be further studied to prevent the rapid loss of this new drug.

4 MODE OF DELIVERY

4.1 Rifampicin (RIF) Delivery

4.1.1 S-SNEDDS

RIF has a poor solubility and bioavailability, while also causing skin microbiome modification and hepatotoxicity (Hakkimane, 2018). In the acidic system, RIF is hydrolyzed to 3-formylrifamycin SV and 1-amino-4-methylpiperazine and in alkaline environment, it is autoxidized into oxidized species like inactive rifampicin quione (Mishra, 2019). To solve this problem, the self-nanoemulsifying drug delivery system (SNEDDS), a lipid-based nanocarrier, is suggested, since it can improve rifampicin's solubility, bioavailblity, and stability (Verma, 2015). SNEDDS can possibly increase the penetration of nanocarrier-based drug through the intestinal mucosa (Hussain, 2019).

Recently, solidified SNEDDS (S-SNEDDS) was developed from the liquid SNEDDS to improve oral bioavailability. It is also an effective dispersible nanoemulsion when contacting with gastric fluid (Hussain, 2019). In the experiment, placebo

SNEDDS performed a stable nano-emulsification after reconstituting with distilled water and an improved permeation across rat intestinal membrane (Hussain, 2019). The dissolution rate was also promoted, as the solid adsorbent increased the specific surface area.

Generally speaking, rifampicin's solubility, bioavailability, and the oral penetration through intestinal mucosal membrane are likely to be improved by SNEDDS, while the S-SNEDDS furthers the benefits of this delivery system.

4.2 Bedaquiline (BDQ) Delivery

4.2.1 Oral Tablets

Currently, BDQ is delivered by oral as tablets, but since it is a novel drug, the mode of delivery is still left for exploration. Two possible routes, inhalation and injection, are discussed below.

4.2.2 Inhalation Delivery

Since TB is a disease in lungs, inhalation might be a target-specific method, with short duration treatment and minimize the side effects caused by oral delivery (Rawal, 2018). The delivery is carried out by nanoparticles (NPs), which are small enough to be easily uptaken by alveolar macrophages (Paranjpe, 2014).

BDQ NPs in the study shows positively charged behavior, facilitating high uptake by the negatively charged sialic acid on alveolar macrophages (Rawal, 2018). No toxicity on cells or organs was shown by the NP formulation, and NP formulation is likely to reduce BDQ dosing frequency comparing to dry powder inhalation (DPI) and oral delivery (Rawal, 2018).

4.2.3 Injectable Delivery by Long-Acting Injectable (LAI) Formulations

LAI formulations are considered to enhance the latent tuberculosis infection (LTBI) completion rates (Swindells, 2018). LAI formulations could also be more convenient for children than oral daily formulations.

To be suitable for LAI, the antibiotic typically has a low aqueous solubilty, which prevents immediate dissolution and release of the drug, and a relatively long pharmacokinetic (PK) elimination half-life (Park, 2013). BDQ, which has a low minimum inhibitory concentration and a long half-life, would probably fits the standards (Kaushik, 2019).

LAI formulations for BDQ can help to overcome poor oral bioavailability, ease the toxicity of BDQ, and reduce drug to drug interactions (Kaushik, 2019). In the study of Kaushik et al., a single dose of long-acting BDQ resulted in apparent duration of bactericidal property, which could last more than 12 weeks, meaning that the plasma BDQ levels were above the MIC for at least 12 weeks after administration. The bactericidal activity of a single injection during those 12 weeks was also significantly more active than the daily oral regimen of same total dose (Kaushik, 2019). This would make possible that two injections of long-acting BDQ, which are administrated 4 weeks apart, can result in the same effect as the WHO-recommended LTBI treatment regimens.

5 DRUG ECONOMICS

5.1 Rifampicin

Before figuring out the medical cost, we have to know the optimum dosage of the drug. Usually, rifampicin is given 10mg/kg (or 600mg) once a day due to a consideration of economics and toxicity (Van Ingen, 2011). However, this dosage might be suboptimal and result in a lower end on the dose-response curve (Boeree, 2015), which is partially responsible for the MDR-TB (Hu, 2015). A new research suggested that 32mg/kg of daily rifampicin is safe and effective at severely-ill patients (Seijger, 2019).

According to Global Drug facility, the price of 600mg daily rifampicin for 25 days is ranged from $5.44 to $14.85. If we apply the new dosage, 32mg/kg daily dosage, the price would be higher, and even tripled.

To treat active TB, rifampicin is taken with ethambutol daily for 2 months and then a combination of rifampicin and isoniazid is implemented for 7 months (Centers for Disease Control and Prevention, 2020). This means that rifampicin alone would be taken each day for 9 months in total, which is already expensive. For latent TB infection, rifampicin is used daily for 7 months in total.

5.2 Bedaquiline

The price of BDQ for a month, comparing to rifampicin, is even higher. The cost of BDQ for 6 months is around $900, $3,000, and $30,000 in low-

income, middle-income, and high-income countries respectively (World Health Organization, 2016).

6 CONCLUSION

6.1 Summary

To sum up, rifampicin and bedaquiline are both bactericidal, while rifampicin inhibits transcription and bedaquiline inhibits the ATP synthesis. As rifampicin is a major component in the most common multidrug regimen, the rifampicin resistance has been severe (Dorman, 2018). In 2015, 125,000 rifampicin-resistant were identified while the estimated number was 580,000 (World Health Organization, 2016). To mitigate the resistance, a maximum rifampicin dosage was found to be 32mg/kg daily (Seijger, 2019), but an optimum dosage should be further investigated. The increased dosage might lead to an even higher medical cost of rifampicin. In contrast, since bedaquiline is novel and has a special mechanism of inhibiting ATP synthesis, less resistance to the drug has appeared (Preiss, 2015). Bedaquiline resistance should be studied and regulated strictly because it is one of the few treatments for XDR-TB (Karmakar, 2019). A limitation of BDQ is that it can only be used for MDR-TB, and it is also expensive ($900 for 6 months treatment in low-income areas) for underdeveloped countries to afford (World Health Organization, 2019). Bedaquiline is a novel drug with significant potential, as it has the opportunity to be administrated by inhalation or long-acting injection. BDQ can directly reach lungs, the site of TB injection, by inhalation method (Rawal, 2018), while long-acting injection enables BDQ to be administrated once a week, improving the completion rate (Kaushik, 2019).

6.2 Evaluation and Future Work

Although oral rifampicin is already mature, it can be improved by new delivery systems such as SNEDDS, so further investigation is still in need. Generally speaking, rifampicin is currently irreplaceable, but it requires solutions to the drug resistance and new strategies to improve its quality, while bedaquiline is a highly critical for treating MDR-TB and XDR-TB, and its delivery methods are still left for explorations.

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