Editorial

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Future Medicinal Chemistry

Rifamycins: do not throw the baby out with the bathwater. Is rifampicin still an effective anti-tuberculosis drug?

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"It safe to say that without this remarkable natural product, the fight against TB would have been much more arduous in the last 50 years, and rifampicin still serves as an effective tool to tackle the TB pandemic."

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The role of rifampicin in tuberculosis treatment

Despite recent additions to the antitubercular armory [1] and the testing of completely novel regimens [2,3], rifampicin remains a cornerstone of tuberculosis (TB) drug therapy. In combination with isoniazid, ethambutol and pyrazinamide and given over 6 months (all four for 2 months, followed by rifampicin and isoniazid for 4 months), rifampicin (together with isoniazid) forms the basis of first-line treatment for this global disease. Rifampicin has been placed on the WHO List of Essential Medicines, as it is deemed to be essential for human health [4]. The potential global market for the WHO and International Union Against Tuberculosis and Lung Disease's recommended rifampicin-containing four-drug fixed-dose combination tablet (rifampicin 150 mg, isoniazid 75 mg, pyrazinamide 400 mg and ethambutol 275 mg) is more than 300 million tablets per year, although more than 75% of all rifampicin formulations used in healthcare services worldwide are still given as single-drug tablets [5].

Historical notes

Rifampicin is a semisynthetic therapeutic agent derived from the rifamycins, a family of ansa antibiotics produced by *Amycolatopsis rifamycinica* that were discovered in 1957 at Lepetit pharmaceutical company [6]. Several metabolites (rifamycins A, B, C, D, E, S and SV) were extracted from the culture broths of this Gram-positive bacterium, and rifamycin B was found to exhibit exceptional sterilizing activity against Gram-positive cocci and *Mycobacterium tuberculosis*. However, it soon became apparent that rifamycin B was ill-suited for clinical use. This natural product was chemically unstable, could only be administered intravenously in hospitals to achieve its desired pharmacological effects and exhibited a poor pharmacokinetic profile [6].

Various unsuccessful attempts were made in the late 1950s and early 1960s to address the issues related to rifamycin B treatment regimens. Eventually, in 1965, the Sensi group developed rifampicin, the hydrazone derivative of rifamycin B, as an oral formulation. Rifampicin (also known as rifampin) retained the excellent bactericidal properties of rifamycin B and had clear advantages in terms of administration routes (e.g., allowing simpler peroral administration) and dosage regimen control, as it allowed patients to better engage in medicine management and achieve high levels of therapy compliance [6].

However, the successful use of rifampicin in the treatment of TB for the past 65 years is associated with a number of shortfalls. These include evolution of drug resistance if used in monotherapy (actual or monotherapy resulting from insufficient exposure to combination drugs), side effects from long-term use (including nausea, vomiting, diarrhea and loss of appetite and hepatoxicity) and induction of metabolic activity of the CYP450 family of enzymes.

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Rifampicin mode of action & design of new analogs

Rifampicin binds to and blocks the exit channel of the RNA polymerase, causing lethal malfunctions in the bacterial transcription machinery. Antimicrobial resistance in strains treated with rifampicin is due to mutation in a 81-base pair region of the *rpoB* gene, termed the rifampicin resistance-determining region (RRDR). This results in modifications or deletions of amino acid sequence present in the chains of the β-subunit of RNA polymerase (RNAP), thus altering the chemical space of the binding site of rifampicin, inhibiting interaction with its molecular target [7].

The type of chemical interactions between rifampicin and RNAP binding site are well documented. The hydrogen bonds formed by the hydroxyl groups at C1, C8, C21, C23 and the acetoxy-protected oxygen at C25 of rifamycin's long chain ('handle') with key polymerase residues are crucial for the biological activity of the ansa antibiotics. More recently, it was found that the C3 rifampicin tail (e.g., a 4-methyl-1-piperazinyl iminomethyl unit) might have a less significant role in establishing interactions with the binding pocket but be more involved in enhancing permeability and water solubility of these compounds, although this is still a matter of debate [8]. Indeed, there are several known active rifamycins (rifabutin-like analogs, benzoxazinorifamycins) whose C3 tails are not capable of interactions with the key residue E445 and are directed toward the Σ-finger of RNAPs. These crucial interactions between the rifamycin handle and RNAP amino acid residues must be taken into account when designing new rifamycin analogs in order to maximize receptor–ligand binding affinity and maintain appropriate levels of antibiotic and sterilizing activities. Moreover, the fine-tuning process of improving the drug metabolism and pharmacokinetic profiles of novel rifamycin analogs, while retaining high antitubercular efficacy, should include chemical modifications of the substituents (tails) appended to the naphthoquinone structure (at carbon 3 or 2) of the ansa antibiotics. Modifications of the C3 tails of rifampicin can also lead to analogs with enhanced antibiotic activity against rifampicin-resistant *M. tuberculosis* strains bearing mutations in the RRDR sequence [8].

Rifampicin resistance in *M. tuberculosis*

TB pathophysiology is complex. The lungs of patients with TB infections are characterized by the presence of granulomatous lesions, including nonvascular caseous (cheese-like) granulomas, which are inhabited by nonreplicating (often drug-tolerant persistent) bacilli. The canonical cocktail of drugs recommended for TB treatment is active against actively replicating bacilli, but ineffective against the nonreplicating bacteria found in the necrotic center of caseous granulomas. The rifamycins are one of the few classes of drugs capable of penetrating and sterilizing caseum lesions in necrotic areas [9]. The central role of rifampicin in TB treatment regimens makes the risk of evolving resistance a major cause for concern. Resistance is driven by exposure to inadequate drug, which may be the result of pharmacological considerations. Importantly, the long course of treatment (6 months for drug-sensitive TB) and unpleasant side effects makes it difficult for patients to adhere to the regimen. Furthermore, the type of formulations for this medication available on the market, for example as single-drug tablets in certain geographical areas and settings (e.g., Eastern Europe, penitentiary detention centers), might exacerbate the emergence of resistance.

Drug resistance in *M. tuberculosis* occurs through mutation or deletion, as there are no mobile genetic elements; thus resistance to rifampicin can be detected through analysis of the RRDR sequence and this emerges at a mutation rate of $10^{-9}/10^{-10}$ mutations per cell division, compared with $10^{-6}/10^{-7}$ for isoniazid [10,11]. For this reason, rifampicin resistance serves as a surrogate for multidrug resistance in practice. The latest WHO data indicate that there are 0.5 million new cases of multidrug-resistant TB per year [12], highlighting the vulnerability caused by reliance on a limited treatment portfolio.

The advent of new antitubercular compounds does not mean we should throw the baby out with the bathwater; rather, we need to consider more carefully how established drugs are applied. In recent years there has been a re-evaluation of the dosing regimen used for rifampicin. Led by Boerre and colleagues in the PanACEA consortium (http://panacea-tb.net/) [13], researchers have accrued evidence for an increase in the optimized dose, from 20 to 40 mg/kg. The original lower dose was a pragmatic choice in the 1960s, when rifampicin was expensive and production limited. Similarly, a recent study by the American AIDS Clinical Trials Group and the Tuberculosis Trials Consortium has demonstrated effective treatment shortening with rifapentine, a close analog of rifampicin [14].

Final remarks

It is apparent that the benefits of using rifampicin for TB treatment outweigh its pitfalls, although there is an increase in drug-resistant TB strains. It safe to say that without this remarkable natural product, the fight against TB would have been much more arduous in the last 50 years, and rifampicin still serves as an effective tool to

tackle the TB pandemic. As it stands, this active pharmaceutical ingredient remains a key element of first-line TB drug regimens and appears to have more aces up its sleeve. In fact, adjustments of the rifampicin dose regimen or chemical alterations of its structural framework might allow this drug, or novel derivatives, to be successfully employed in TB treatment for many years to come.

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References

1. Working Group on New TB Drugs. New drugs for TB. www.newtbdrugs.org/
2. McHugh TD, Honeyborne I, Lipman M, Zumla A. Revolutionary new treatment regimens for multidrug-resistant tuberculosis. *Lancet Infect. Dis.* 19(3), 233–234 (2019).
3. Conradie F, Diacon AH, Ngubane N *et al.* Treatment of highly drug-resistant pulmonary tuberculosis. *N. Engl. J. Med.* 382(10), 893–902 (2020).
4. World Health Organization. World Health Organization model list of essential medicines: 21st list 2019. World Health Organization, Geneva (2019). apps.who.int/iris/handle/10665/325771
5. Norval PY, Blomberg B, Kitler ME, Dye C, Spinaci S. Estimate of the global market for rifampicin-containing fixed-dose combination tablets. *Int. J. Tuberc. Lung Dis.* 3(3 Suppl. 11), S292–S321 (1999).
6. Sensi P. History of the development of rifampin. *Rev. Infect. Dis.* 5(Suppl. 3), S402–S406 (1983).
7. Campbell EA, Korzheva N, Mustaev A *et al.* Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell* 104, 901–912 (2001).
8. Zloh M, Gupta M, Parish T, Brucoli F. Novel C-3-(N-alkyl-aryl)-aminomethyl rifamycin SV derivatives exhibit activity against rifampicin-resistant *Mycobacterium tuberculosis* RpoBS522L strain and display a different binding mode at the RNAP β-subunit site compared to rifampicin. *Eur. J. Med. Chem.* 225, 113734 (2021).
9. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. *Int. J. Tuberc. Lung Dis.* 4(9), 796–806 (2000).
10. Billington OJ, McHugh TD, Gillespie SH. Physiological cost of rifampin resistance induced *in vitro* in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 43(8), 1866–1869 (1999).
11. O'Sullivan DM, McHugh TD, Gillespie SH. The effect of oxidative stress on the mutation rate of *Mycobacterium tuberculosis* with impaired catalase/peroxidase function. *J. Antimicrob. Chemother.* 62(4), 709–712 (2008).
12. World Health Organization. WHO consolidated guidelines on tuberculosis, module 4: treatment – drug-resistant tuberculosis treatment (2020). www.who.int/publications/i/item/9789240007048
13. Te Brake LHM, de Jager V, Narunsky K *et al.* Increased bactericidal activity but dose-limiting intolerability at 50 mg·kg^{-1} rifampicin. *Eur. Respir. J.* 58(1), 2000955 (2021).
14. Dorman SE, Nahid P, Kurbatova EV *et al.* Four-month rifapentine regimens with or without moxifloxacin for tuberculosis. *N. Engl. J. Med.* 384(18), 1705–1718 (2021).