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Research progress of pyrazinamide in the treatment of drug-resistant tuberculosis

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Abstract: *Pyrazinamide (pyrazinamide) is a nicotinamide analog. As a unique treatment for tuberculosis, antimicrobial drugs have shortened the treatment time, improved the efficiency of treatment, and reduced the recurrence rate, which is different from other common anti-tuberculosis drugs by inhibiting multiple targets, such as energy production, reverse translation, and survival pantothenate/coenzyme A. Therefore, it is used as an irreplaceable part of the second-line treatment of multidrug-resistant tuberculosis. In recent years, a combination of multiple anti-tuberculosis drugs, such as pyrazinamide and rifampin, has been found to improve the radical cure rate of tuberculosis. This review discusses the recent developments in pyrazinamide.*

Keywords: *Pyrazinamide; drug-resistant tuberculosis; antibiotic; mycobacterium tuberculosis*

1. Introduction

In the Global Tuberculosis Report 2022, the World Health Organization noted that an estimated 10.6 million people are infected with TB worldwide, including 1.3 million of them (including 167,000 people infected with HIV). Tuberculosis (TB) is the second most important infectious killer after COVID-19. Currently, the most important factor affecting cure and mortality rates of tuberculosis is drug resistance. An estimated 191000 people (range: 119000 – 264000) died due to MDR/TB in 2021. Therefore, the rational use of anti-resistant tuberculosis drugs such as pyrazinamide plays an important role in the control and treatment of diseases. Pyrazinamide was first chemically synthesized in 1936 and was found to be resistant to tuberculosis in 1952[1-3].However, McDermott et al. proved that pyrazinamide and isoniazid (INH) combination has high sterilization activity; currently, pyrazinamide is an important part of multidrug-resistant TB treatment internationally and is also a clinical trial of any new scheme in combination with TB candidate [4-6]. Pyrazinamide is a first-line drug released by the WHO. This paper summarizes the recent research progress of pyrazinamide in the treatment of drug-resistant tuberculosis and provides new ideas for exploring the application scope of pyrazinamide therapy and exploring the more accurate and effective drug combination use of drug-resistant tuberculosis therapy[7].

2. Drug resistance mechanisms in Mycobacterium tuberculosis

2.1. Mechanism of resistance to rifampin

The mechanism of action of rifampin is binding to the DNA-dependent RNA polymerase of M. tuberculosis, thereby inhibiting its activity and hindering the DNA replication process, as well as transcription and translation, thus inhibiting DNA and protein synthesis and leading to the death of M. tuberculosis. However, since rifampin acts on the rpoB gene encoding the β subunit of RNA polymerase, more than 95% of rifampin resistance mutations are associated with mutations in the rpoB gene[8]. The specific mechanism of action is that part of rifampin-resistant Mycobacterium tuberculosis is affected by mutations in the rpoB gene of the RNA polymerase β subunit, resulting in rifampin being unable to bind to RNA polymerase with higher affinity, resulting in the emergence of rifampin resistance [9], in which the most common mutated codon is the serine at position 531 to leucine [10]. Many studies have been conducted to develop a mechanism and weight arrangement system for this mutation to improve the efficiency of drug discovery [11].

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2.2. Mechanism of resistance to isoniazid

Isoniazid (IFN) is mainly activated by the bacterial peroxide-catalase KatG (encoded by Rv1908c) to form the isoniazid-NAD complex. Isoniazid-NAD binds to enoyl-ACP reductase (InhA, Rv1484), which is involved in the elongation of long-chain fatty acids, and is an important link in the branching acid synthesis pathway. By interfering with InhA activity, isoniazid leads to a decrease in mycobacterionic acid synthesis and inhibition of cell wall biosynthesis, leading to damage to the cell wall function,thereby affecting bacterial survival.[12] However, most drug-resistant bacteria of Mycobacterium tuberculosis are related to mutations in katG, mamA-inhA, oxyR-ahpC, kasA, and ndh, and mutations in katG make it difficult to produce catalase, which makes isoniazid and Mycobacterium tuberculosis unable to act. The overexpression of the InhA gene also causes excessive synthesis of branching acid, while the ketG gene mutation is mainly caused by mutation of the AphC gene[13-14].

2.3. Mechanism of resistance to streptomycin

Streptomycin (SM) interacts with the surface of bacterial cells through ionic bonds, enters the periplasmic space, and then is transported to the cytoplasm through some membrane channels. Once it enters the cytoplasm of Mycobacterium tuberculosis, it binds to the 30S ribosome with high affinity to inhibit the synthesis of related proteins[15]. The rpsl gene of the S12 ribosomal protein is encoded by approximately 55.5% of streptomycin-resistant strains, and approximately 15% of streptomycin-resistant strains have mutations in the rrs gene encoding 16 S rRNA. The reason for the limited effect of streptomycin and Mycobacterium tuberculosis is caused by the mutation of rpsL and rrs genes, which affects the inhibition of protein synthesis, leading to the emergence of drug resistance, and the clinical production of streptomycin-resistant bacteria is significantly related to these two genes [16-17].

2.4. Mechanism of resistance to ethambutol

Ethambutol (EMB) mechanism of action mainly by inhibiting arabinosyltransferase, inhibit arabinose group polymerization into the arabinogalactan and lipid cell wall, interfere with cell wall biosynthesis, destroy the integrity of the cell wall of mycobacterium tuberculosis, resulting in the mycobacterium tuberculosis death, and mycobacterium tuberculosis resistance mutations is some of the arabinogalactcan biosynthesis and biological activity of related gene mutations[18-19]. Ethambutol resistance in Mycobacterium tuberculosis is mainly associated with mutations in the embCAB locus, with embB mutations being the most dominant [20].

2.5. Pyrazinamide Resistance Mechanisms in Mycobacterium tuberculosis

Despite its critical role in TB therapy, emerging pyrazinamide resistance poses a significant challenge. Approximately 50-85% of multidrug-resistant TB (MDR-TB) strains exhibit pyrazinamide resistance, primarily linked to mutations in the pncA gene encoding pyrazinamidase. Pyrazinamidase catalyzes the conversion of pyrazinamide to active POA. Over 300 pncA mutations have been identified, including frameshift mutations, missense mutations, and promoter region alterations, leading to impaired enzyme activity or expression. Notably, a 2017 multiple genome analysis of Mycobacterium tuberculosis revealed that many of pyrazinamide-resistant isolates harbored pncA mutations. Mutations in rpsA were only found in resistant strains that harbored critical pncA mutations, which are known to cause pyrazinamide resistance. mutations were found in the fas gene in resistant strains without pncA mutations. However, no significant association with PZA resistance was found[21-24].

Additionally, studies identified mutations in the panD gene (encoding aspartate decarboxylase) as a novel resistance mechanism. panD mutations reduce bacterial susceptibility to POA by altering coenzyme A biosynthesis, thereby diminishing the drug's disruption of energy metabolism[25]. Furthermore, efflux pump overexpression (e.g., Rv1258c) has been correlated with pyrazinamide tolerance in vitro, suggesting a role in low-level resistance[26]. These findings underscore the complexity of resistance mechanisms and highlight the need for comprehensive molecular diagnostics targeting multiple genetic loci.

3. The role and mechanism of pyrazinamide against drug-resistant tuberculosis

Pyrazinamide is an important anti-tuberculosis drug that can treatment for 9-12 months greatly shortened to 6 months, and its bactericidal activity is mainly due to its bactericidal activity, which can

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kill Mycobacterium tuberculosis, and its antibacterial effect in an acidic environment has its antibacterial effect [27-28]. Pyrazinamide is converted within Mycobacterium tuberculosis into its active partial pyrazinic acid (POA). Low pH outside of the mycobacteria favors the accumulation of POA within the mycobacteria. POA accumulates within Mycobacterium tuberculosis to a certain amount to destroying the intracellular acid and base balance of Mycobacterium tuberculosis. It also affected the energy metabolic activity of Mycobacterium tuberculosis. And since pyrazinamide is a nicotinamide analog, while a nicotinamidase is often present in the bacterial intracellular. Nicotinamidase can convert nicotinamide to niacin. Then niacin is biosynthesized into nicotinamide dinucleotide (NAD) to participate in the basal metabolism. Whereas the nicotinamidase of Mycobacterium tuberculosis is localized in the cytoplasm. Since pyrazinamide has a similar structure to nicotinamide, the nicotinamidase of Mycobacterium tuberculosis can also convert pyrazinamide to its active form, pyrazinate. This favors the massive aggregation of POA within M. tuberculosis. Thus killing the Mycobacterium tuberculosis. For some multidrug-resistant Mycobacterium tuberculosis, pyrazinamide has an unexpected effect. And the treatment of children with rifampicin-resistant tuberculosis,isoniazid 300 mg, pyrazinamide 750 mg, ethambutol 750 mg, and linezolid 300 mg (8 h once), has a good treatment effect [29-33].

4. Effect of pyrazinamide and other anti-tuberculosis drugs

In 1954, researchers found that pyrazinamide combined with other bactericidal substances (isoniazid) could effectively kill tuberculosis [34]. In recent years, studies have found that drug-sensitive tuberculosis patients treated with streptomycin, isoniazid, and pyrazinamide for 9 months had a 2-year recurrence rate of only 5–6% [35-36]. In addition to its effects in combination with some classic anti-tuberculosis drugs, pyrazinamide also enhances the activity of new and research drugs (such as bedaquoline, deramani, and putomanil)[37-39]. It is of concern that different doses of pyrazinamide differ in combination with different doses of drugs, including rifampin and pyrazinamide, where the best anti-tuberculosis effect is observed when both are high doses[40].In the process of exploring pyrazinamide for the better treatment of resistant tuberculosis, researchers have also found that some newly developed drugs (such as diaryl quinoline beta quinoline) and pyrazinamide have synergistic activity. If the organism remains sensitive to pyrazinamide, the use of pyrazinamide, including new drugs, can greatly improve its efficacy [41-42]. A recent retrospective study confirmed that pyrazinamide combined with a new generation of fluoroquinolones and second-line injection of anti-tuberculosis drugs significantly increased the rate of sputum culture at 3 months, 2 years treatment success rate also increased, while a treatment regimen including pyrazinamide and ethambutol can significantly reduce the probability of treatment failure or death[43,44].

5. Advancements in Pyrazinamide Delivery Systems

To overcome pharmacokinetic limitations and hepatotoxicity, novel drug delivery strategies have been explored:

5.1. Nanotechnology-Based Approaches

Liposomal encapsulation of pyrazinamide enhances intracellular drug accumulation in macrophages. A murine study demonstrated that liposomal pyrazinamide achieved 3-fold higher lung concentrations compared to conventional formulations, reducing treatment duration by 30%[45]. Recent research has concentrated on employing starch-derived bulk and nanopolyurethanes (SBPUs and SNPUs) as drug delivery systems (DDS) to load and deliver first-line anti-tuberculosis drugs (ATDs), including isoniazid, rifampicin, pyrazinamide, and streptomycin, aiming to mitigate or eliminate their adverse effects. Notably, anti-TB activity assays against Mycobacterium tuberculosis H37Rv demonstrated that streptomycin-loaded SNPU4i exhibited 42-fold higher efficacy compared to free streptomycin, while isoniazid-loaded SNPU7i showed a 7-fold increase in potency relative to native isoniazid.[46]

5.2. Inhalable Formulations

Dry powder inhalers (DPIs) facilitate direct pulmonary delivery and minimize systemic exposure. Phase trials of pyrazinamide-DPI formulations reported that add-on combined anti-TB DPI therapy to the standard oral anti-TB treatment did not increase MTB sputum culture conversion at two months of treatment. However, the percentage of patients exhibiting cough in the study group was significantly lower than that in the control group two months after treatment. A reduction in cough may indicate an

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adequate response to treatment and result in a decreased risk of infection transmission. Researchers have developed triple combination spray-dried inhalable formulations composed of antitubercular drugs, pretomanid, moxifloxacin, and pyrazinamide (1:2:8 w/w/w) alone (PaMP), and in combination with an aerosolization enhancer, L-leucine (20 % w/w, PaMPL). The in vitro efficacy studies demonstrated that the triple combination formulation exhibited more prominent antibacterial activity with a minimum inhibitory concentration (MIC) of 1 µg/mL against the MTb H37Rv strain compared to individual drugs. The triple combination of pretomanid, moxifloxacin, and pyrazinamide as an inhalable dry powder formulation may potentially improve treatment efficacy with reduced systemic side effects in patients suffering from latent and multidrug-resistant TB. This approach may benefit patients with hepatic comorbidities.[47-49]

6. Developing Novel Drug Combinations

In light of the global increase in drug-resistant tuberculosis, considerable attention is being directed toward the development of novel drug combinations that offer reduced treatment duration and improved toxicity profiles. The targeting of nicotinamide adenine dinucleotide (NAD) biosynthesis is recognized as a promising approach for addressing drug-susceptible, drug-resistant, and latent tuberculosis (TB) infections.[50]

7. Clinical Management Strategies for Pyrazinamide-Resistant TB

The WHO 2022 guidelines recommend pyrazinamide susceptibility testing for all MDR-TB cases. For confirmed resistance, optimized regimens include:

BPaL regimen: Bedaquiline (B), pretomanid (Pa), and linezolid (L) showed 89% success rates in pyrazinamide-resistant populations during the Nix-TB trial[51].

High-dose rifampicin combinations: Co-administration of 35 mg/kg rifampicin with moxifloxacin compensates for pyrazinamide resistance by enhancing sterilizing activity[52].

Host-directed therapies (HDTs): Adjuvants like metformin enhance pyrazinamide efficacy by modulating host autophagy pathways, even in resistant strains[53].

8. Conclusion

With the rapid advancement of molecular biology and related disciplines, research on the mechanism of action of pyrazinamide in tuberculosis has progressed significantly. Molecular biological analysis of the structure of relevant MTB proteins has been utilized to elucidate more comprehensive and advanced mechanisms of drug resistance development, leading to the creation of novel drugs and improved combination therapies with pyrazinamide. However, there is growing concern regarding the global emergence and rapid increase in pyrazinamide-resistant Mycobacterium tuberculosis. In Mycobacterium tuberculosis, pyrazinamide resistance gene mutations, particularly pncA gene mutations, are the most prevalent and occur at various sites, with some strains exhibiting mutations at multiple locations within pncA. Consequently, pncA mutations are widely considered the molecular basis of pyrazinamide resistance in MTB. tuberculosis; thus, the effective prevention of pyrazinamide resistance in MTB. tuberculosis is crucial. Some researchers have also reported that pyrazinamide exhibits hepatotoxicity[54-56]. Therefore, the development of improved, safer, and more effective methods for utilizing pyrazinamide in the treatment of drug-resistant tuberculosis presents a significant challenge, and these issues require further investigation.

To address these challenges, ongoing research is focused on elucidating the genetic and molecular factors that contribute to pyrazinamide resistance and hepatotoxicity, as well as developing new strategies to overcome these obstacles.

One approach involves identifying novel genetic markers that can predict resistance to pyrazinamide. Whole-genome sequencing and targeted gene sequencing have been instrumental in identifying various genetic mutations associated with drug resistance. For example, mutations in the rpsA gene, which encodes the ribosomal protein S1, have been linked to pyrazinamide resistance in some cases. Identifying these genetic markers can facilitate the early detection of resistance and inform more effective treatment regimens.[57]

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Another area of research is aimed at understanding the mechanisms of pyrazinamide's hepatotoxicity. While the precise mechanism remains unclear, it is hypothesized that the metabolism of pyrazinamide in the liver, involving cytochrome P450 enzymes, may contribute to its toxic effects. Studies are underway to investigate the role of specific enzymes and to develop alternative drug formulations that may reduce hepatotoxicity. Additionally, drug-drug interactions that may exacerbate liver damage are being systematically evaluated.

The development of new drug candidates that act on the same or alternative pathways as pyrazinamide is also a priority. For instance, researchers are exploring the potential of nicotinamide adenine dinucleotide (NAD+)-dependent deacylases inhibitors, which could target the same pathway as pyrazinamide but with potentially fewer adverse effects. These novel compounds could provide alternative treatments for drug-resistant tuberculosis, particularly when combined with existing therapies.[58]

Furthermore, the utilization of pharmacokinetic and pharmacodynamic modeling is facilitating the optimization of dosing regimens for pyrazinamide. By elucidating the concentration-time profiles required to effectively eradicate Mycobacterium tuberculosis, researchers can tailor drug regimens to maximize efficacy while minimizing the risk of resistance development and adverse effects.

In conclusion, while the emergence of pyrazinamide resistance presents a significant challenge in the treatment of drug-resistant tuberculosis, substantial progress is being made in understanding the molecular basis of resistance and hepatotoxicity. These advancements are paving the way for the development of new diagnostic tools, safer drug formulations, and more effective combination therapies. As such, the future of pyrazinamide treatment in drug-resistant tuberculosis appears promising, with the potential to significantly improve patient outcomes and combat the global tuberculosis epidemic.

However, these efforts require continued investment in research and development, as well as collaboration between academia, industry, and public health agencies. By addressing the current limitations and exploring new avenues for treatment, the international community can work towards eradicating drug-resistant tuberculosis and ensuring the efficacy of pyrazinamide as a critical component of tuberculosis therapy.

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