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Development of a three component complex to increase isoniazid efficacy against isoniazid resistant and nonresistant *Mycobacterium tuberculosis*

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ABSTRACT

The bacterium responsible for causing tuberculosis has evolved resistance to antibiotics used to treat the disease, resulting in new multidrug resistant *Mycobacterium tuberculosis* (MDR-TB) and extensively drug resistant *M. tuberculosis* (XDR-TB) strains. Analytical techniques ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), Fourier Transform-Ion Cyclotron Resonance with Electrospray Ionization (FT-ICR/ESI), and Matrix Assisted Laser Desorption Ionization–Mass Spectrometry (MALDI-TOF–MS) were used to study different aspects of the Cu(II)–polyethylene glycol (PEG-3350)–sucrose–isoniazid and Cu(II)–polyethylene glycol (PEG3350)–glucose–isoniazid complexes. The Cu(II) cation, sucrose or glucose, and the aggregate formed by PEG primarily serve as a composite drug delivery agent for the frontline antibiotic, however the improvement in MIC values produced with the CU–PEG–SUC–INH complex suggest an additional effect. Several Cu–PEG–SUC–INH complex variations were tested against INH resistant and nonresistant strains of *M. tuberculosis*.

Tuberculosis is a bacterial disease caused by *Mycobacterium tuberculosis* which results in a chronic bloody cough among other symptoms, and currently infects approximately one out of three individuals worldwide. Antibiotic resistance has emerged due to spontaneous mutations within the genome of the bacterium causing the development of resistance mechanisms to antibiotics which has resulted in extremely difficult cases to treat. Multidrug resistant tuberculosis (MDR-TB), extensively drug resistant tuberculosis (XDR-TB), and totally drug resistant tuberculosis (TDR-TB) cases have become a global threat.[1]

A person infected with an active *M. tuberculosis* infection undergoes 6–9 months of directly observed treatment short-course (DOTS) using a combination of the frontline TB drugs isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB). MDR-TB refers to strains of *M. tuberculosis* that are resistant to INH and RIF, while XDR-TB strains are resistant to INH, RIF, and the second-line TB drugs fluoroquinolines and at least one of the injectable aminoglycosides. MDR-TB cases can cause treatment to lengthen up to an additional 18 months and pose more harmful side effects due to the use of more toxic second-line drugs. Use of ineffective treatment regimens and difficulty in detecting antibiotic resistance among bacterial strains has exacerbated the evolution of drug resistant *M. tuberculosis*.[2]

A limiting factor for effectiveness in TB treatment regimens is the lengthy treatment time which affects a patient's adherence, therefore affecting the progression of infection and ultimately development of antibiotic resistance. Standard treatment for latent tuberculosis infections (LTBI) requires 6–12 months of antibiotic therapy using INH alone, but shorter therapies have been suggested in order to increase adherence to treatment. LTBI standard treatment has been compared to the alternative combination therapy of INH and RIF for a duration of 3 months. Results suggested that both treatments are equal in terms of effectiveness, toxicity and mortality, but shorter course therapies using both INH and RIF could result in greater compliance by patients.[3] Another limiting factor for treating tuberculosis, particularly with resistant cases, is adverse side effects. A study by Reves et al.[4] evaluating a treatment plan for INH resistance or intolerance which consisted of RIF, PZA, and EMB found it to be effective but highly toxic,

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resulting in 26% of participants stopping treatment due to adverse reactions such as hepatotoxicity.[4]

The need for rapid and accurate diagnosis of TB infections is crucial for transmission control and has resulted in the development of improved diagnostic tests such as *M. tuberculosis* direct nucleic acid amplification testing (MTD), which is over 95% accurate in terms of sensitivity and specificity. Due to its high accuracy and rapid diagnosis time, implementing the use of MTD could help hospitals and clinics avoid unnecessary costs when diagnosing infections and investigating contact transmission. The use of MTD was found to be particularly cost effective when diagnosing HIV infected patients, the homeless, and substance abusers.[5]

Cases of MDR-TB are projected to rise, and testing for resistance to specific antibiotics is key in order to reverse this trend. Even though the highest percentage of resistant *M. tuberculosis* isolates are resistant to INH, testing for INH-monoresistance would not help decrease the number of cases on a large scale. It has been suggested that implementing RIF-resistance tests alongside rapid tests for TB would have more benefits, such as cost-effectiveness and a predicted reduction in MDR-TB cases by 30%.[6] Strains with RIF resistance have resulted from mutations affecting RNA polymerase. The development of the polymerase chain reaction-single-strand conformation polymorphism strategy can detect RIF resistance. Determining if a strain is resistant to a particular antibiotic can help physicians choose more effective treatment regimens and decrease the chances of treatment failure and likelihood of developing further resistance.[7]

Mutations in the *M. tuberculosis* genome causing resistance to INH are most commonly found in the katG gene followed by the promoter in inhA. However, the mutated gene or genes responsible for causing resistance has not been determined in several isolates. A study by Shekar et al.[8] identified 60 genes causing INH resistance for reasons such as, but not limited to: intervening with cell wall and cell processes, metabolism, respiration, and lipid metabolism. The ratio of non-synonymous mutations to synonymous mutations was also found. This ratio helps to determine how quickly genes are evolving which aids in finding targets to develop new antibiotics.[8]

A study by Machado et al.[9] testing drug susceptibility of isolates from Lisbon, Portugal, an area with a high burden of resistant strains of TB, found that when a mutation occurred in both the regulatory and coding region of inhA, there was a high level of resistance to both INH and EMB. Treatment regimens of strains similar to this strain eliminate first-line antibiotic options for treatment and potentially lead to using more toxic, costly second-line drugs.[9]

Adding drug efflux inhibitors to anti-tuberculosis drugs has been suggested to increase efficacy of drugs by increasing the amount of the drug within the bacterial cells or by affecting the immune response of the cell. A study by Gupta et al.[10] found that when verapamil, a drug efflux inhibitor, was added to bedaquiline and clofazimine, an 8 fold or more reduction of MIC values for both drug resistant and nonresistant *M. tuberculosis* isolates resulted. It was also noted that drug efflux may develop as a resistance mechanism to these drugs.[10]

Quantitative structure activity relationship (QSAR) models have been generated in order to determine structural requirements for advancing and developing new drugs to target TB. A study focusing on QSAR models of fluoroquinolines, a group of synthetic antibiotics derived from nalidixic acid, found that stereochemistry is the most important factor affecting affinity of the drug. Therefore, modification of the stereochemistry of fluoroquinolines could alter their efficacy and lead to the development of new drugs.[11]

PA-824 and bedaquiline are examples of TB drugs that have entered clinical trials since 2004. Early bactericidal activity assessments of PA-824, bedaquiline, moxifloxacin, and PZA have shown the compounds to be safe and effective. The addition of PZA to bedaquiline regimens has resulted in more efficacious antibacterial activity. However, PA-824-PZA-moxifloxacin treatments exhibit higher efficacy than combinations using bedaquiline and are comparable to the standard regimen isoniazid–rifampicin–pyrazinamide–streptomycin.[12]

Bedaquiline has been the only new TB antibiotic to be released to the market in forty years, and it is particularly effective for treating MDR-TB cases. This drug has been shown to significantly shorten culture conversion, and to be twice as effective in curing TB when compared to placebo groups. While participants in studies taking bedaquiline have shown adverse effects, they were not different from ones commonly caused by second-line TB drugs and most often did not result in termination of treatment. More deaths have occurred in bedaquiline recipient groups compared to placebo groups, but causation of these deaths was inconclusive and further data must be obtained.[13] Bedaquiline has been added to background regimens in study trials to determine its safety, tolerability, efficacy, and rate of resistance acquisition. When compared to standard regimens alone, the addition of bedaquiline significantly reduced culture conversion and time to reach sputum culture negativity. Acquisition of drug resistance and incidence of most adverse effects were lower when using bedaquiline in treatment regimens rather than standard treatment alone.[14]

Altering the structural design of the antibiotic spectinomycin has resulted in the low-cost development of a new class of semisynthetic anti-mycobacterial drugs called spectinamides. Previously, spectinomycin has not been used as a treatment for TB due to efflux of the drug. However, with structural modification to the drug, the Rv1258c efflux pump can be avoided and binding to the mycobacterial ribosome can increase. Spectinamides have shown to have safe pharmacological activity, significantly reduce MIC values, and have activity against MDR-TB and XDR-TB strains.[15]

Since its discovery in 1952, INH (see Fig. 1) has served as an important antibiotic in treating *M. tuberculosis* infections and is currently a standard TB drug. The compound was discovered by Bernstein et al.[16] and demonstrated that isonicotinic acid hydrazide and its derivatives had high in vivo activity against *M. tuberculosis*. It was found that the required effective dosage rate was lower than any other compound that had been tested to date.[16] INH is a prodrug that becomes activated through peroxidation by the enzyme katG after entering mycobacterial cells via passive diffusion. INH couples with the intermediate species $NAD^+/NADP^+$ which are key in the mechanism of action of the antibiotic. The drug acts to inhibit cell wall lipid synthesis, inhibit nucleic acid synthesis, and decrease respiration.[17]

Polyethylene glycol-3350 (PEG) is a polymer widely used for medicinal purposes, and has been complexed with antibiotics to alter their delivery mechanism. Multiple polymer compounds used as core shell nanoparticles that have included PEG have shown to increase bioavailability of INH by 28 fold.[18] PEG derivatives of INH have been demonstrated to have lower cytotoxicity and increased residence time at infection sites compared to INH alone. Increasing molecular weights of PEG complexed with INH resulted in higher residence time in the bloodstream.[19]

The potent antimycobacterial activity of Cu(II) has been noted in several studies.[20–22] INH complexes with Cu(II) by binding through oxygen and amine nitrogen atoms.[23] The addition of Cu (II) to INH increases activity against mycobacteria especially under high Fe conditions (8 μg/mL).[24] As demonstrated by the Irving Williams series, the Cu ion has a thermodynamic preference for binding amines over other physiologically relevant cations such as Zn (II), Fe(II), Ca(II), and Mg(II).

The extent of testing disaccharides complexed with TB drugs in order to alter cellular delivery has not been reported in detail in the scientific literature. However, a study by Jain et al.[25] evaluated the