Inhibiting Dihydrofolate Reductase as a Treatment for Tuberculosis


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Abstract: One-third of the world’s population is infected by Mycobacterium tuberculosis (M.tb). Two million people die each year from tuberculosis (TB), the disease caused by this bacterium. TB primarily affects the lungs and is easily transmitted. One way to kill M. tb might be to inhibit the enzyme dihydrofolate reductase (DHFR). DHFR catalyzes the production of tetrahydrofolic acid by transferring a hydrogen ion from NADPH (nicotinamide adenine dinucleotide phosphate) to dihydrofolate, thereby releasing tetrahydrofolic acid and NADP+. Tetrahydrofolic acid is essential to the bacterial’s survival, and is a cofactor that is needed for the synthesis of the DNA base thymine. Isoniazid is one antibiotic already used to treat TB by targeting several TB proteins that are necessary for building Mycobacterium tuberculosis cell walls and inhibiting DHFR. Unfortunately, strains of M. tb are evolving resistance to isoniazid, so next generation antibiotics are needed. A variation of isoniazid could be designed to avoid resistance, and to inhibit DHFR, thereby targeting bacterial DNA synthesis. The Valders SMART team, using rapid three dimensional printing technology, created a physical model of DHFR and a possible inhibitor of tetrahydrofolate production. Supported by a grant from NIH-NCRR-SEPA.

Introduction: Two million people die each year from tuberculosis (TB). The bacterium that causes this disease, Mycobacterium tuberculosis, infects over one third of the world’s population. This study is devoted to modeling a new drug for Trimethoprim, an antibiotic used to treat TB that is losing effectiveness due to drug resistance. We are modeling a possible inhibitor of tetrahydrofolate production that could effectively prevent TB cell multiplication and could possibly be used as an antibiotic to treat the infection.

The Problem: Bacteria are becoming resistant to current drugs, like trimethoprim (Fig. 1). The goal of this research is to find a drug that binds to dihydrofolate reductase (DHFR) (Fig. 2) like trimethoprim does. Isoniazid has the potential to be this alternative drug. The natural process shown to the right, models the normal activity of DHFR in a bacterial cell. The production of tetrahydrofolic acid (THF) allows for the synthesis of DNA that is needed for bacterial cell (TB) growth.

A Novel Approach: Isoniazid is a pro-drug (Fig. 3). It is consumed in its inactive form and then converted to an active form in the liver. The process of this activation allows the potential for drug resistance to develop. The lab of Dr. Daniel Sem hopes to engineer a form of isoniazid that does not require this step, therefore hopefully preventing future drug resistance. The inhibited process modeled to the right shows how isoniazid may prevent THF production. This process is believed to mimic that of trimethoprim which inhibits DNA synthesis in other bacteria. Fortunately, human DNA synthesis would not be inhibited because there is only a 26% sequence alignment between human DHFR and M. tuberculosis DHFR.

Method of Study: Nuclear magnetic resonance, or NMR, is used by scientists to understand the structure and function of proteins. Nuclei of atoms (in this case, nitrogen atoms) have spin when in a magnetic field, and when a second electromagnetic field is applied, the atoms “flip”, and this phenomenon is visualized as an NMR spectrum (Fig. 6), with each backbone amide (N-H) of the protein represented as one “spot” – called a cross-peak. One cross-peak has been expanded to show how it shifts upon a protein binding to an inhibitor. Such data could be used to determine where (and how tightly) a molecule like NADP+ or trimethoprim binds to DHFR. Related experiments can be used to calculate a 3-D structure of the protein-drug complex.

Disease Aspect: TB primarily affects the lungs and can be transmitted by coughing and sneezing. The active infection of tuberculosis has symptoms that include a mild fever, weight loss, night sweats and persisting coughing. The treatment takes place over a six month span and involves a cocktail of four anti-TB drugs. Not everyone that is infected with TB gets sick, which is called latent TB infection. This occurs when the bacteria live in the body but do not cause visible symptoms. At this stage, TB is not contagious, but if untreated it can become active causing the bacteria to multiply. If TB goes untreated, it can be fatal.

Conclusion: Tuberculosis causes two-million deaths every year worldwide. A common antibiotic used to treat other bacterial infections, by inhibiting DHFR, is trimethoprim. Isoniazid, used to treat TB, also inhibits DHFR – but it is loosing effectiveness. It is hoped that a modified version of Isoniazid can be designed, based on structures of trimethoprim bound to DHFR. Isoniazid inhibits THF, restricting production of thymine which inhibits tuberculosis cell replication, therefore killing the TB bacteria. Isoniazid is a pro-drug so it is consumed in an inactive form and then converted to an active form inside the liver. This creates potential to form resistance. Scientists hope to have the modified drug skip this step. The new version could then be used as a treatment for the third of the world population that is infected with tuberculosis.

NMR Binding Analysis: To confirm that a molecule like NADP+, isoniazid, or trimethoprim binds to DHFR, an NMR titration experiment could be done. The spots in this spectrum correspond to amides in a protein (one of each amino acid) – and they change their location in the spectrum when increasing amounts of the binding molecule are added. The different colors represent spectra with more of the binding molecule added.