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A Bio-chemical and Microbiological Appraisal on the Stability of the Second-line Anti-Tuberculosis Drug, Cycloserine in *Mycobacterium tuberculosis*

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Cycloserine ($C_3H_6N_2O_2$) is a second-line drug that is used to treat tuberculosis. Second-line drugs are usually administered to tuberculosis patients when treatment with first-line TB drugs fail or their effect in combination therapies fail or are adverse. Cycloserine has been associated with a number of adverse effects, mostly because of its instability and high levels of toxicity. This article is germane in that it addresses a way of eliminating the instable nature of this drug by studying this drug microbiologically and bio-chemically.

Keywords: Cycloserine, Broth microdilution method, pH, Electon-rich, Electron-deficient.

Cycloserine ($C_3H_6N_2O_2$, Sigma, 2008) is a broad-spectrum second-line antibiotic that is bacteriostatic at the recommended clinical dosage. It is available as, either a capsule (WHO PAR Part 4, 2007) or a white/whitish-yellow powder that is completely soluble in water and partially in ethanol (Official Monographs for Part 1).

The potency, hypersensitivity- (WHO PAR Part 4, 2007) and neurologically-related outcomes (Wolinsky, 1993) of cycloserine on patients has made it difficult to treat multidrug resistant (MDR) tuberculosis (TB) patients cost effectively, because neurologically-affected patients are obligated to monthly neuropsychiatric assessments. Among the psychiatric implications are central nervous system toxicity (convulsions, psychosis, somnolence, depression, confusion, hyperflexia, headache, tremor, vertigo, paresis, or dysarthria), and the less well understood human pregnancy/mother breastfeeding cases on treatment (WHO PAR Part 4, 2007). However, it has also been used to the betterment of the patient (Rishan Singh, personal writing) e.g. to treat renal and hepatic treatment.

In 2008, a study at the Nelson R. Mandela School of Medicine, in the Department of Medical Microbiology, was conducted as part of an ongoing study in assessing *M. tuberculosis* susceptibility to cycloserine using pre-determined laboratory conditions. In a previous study, performed by someone else, cycloserine susceptibility was assessed in $H_{37}R_V$ by evaluating its responses to the effects of increasing pH (6.8, 7.2, 7.5, 7.8, and 8.1) by deriving time-kill curves. The method that was used is briefly described in Kent *et al*. (1992).

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It was observed that a concentration of 32 µg/ml optimally killed the isolates at a pH of 7.2, after 14 days. The control was maintained at pH 6.8. Using the pH of 7.2 and standard laboratory conditions, the minimum inhibitory conditions (MICs) of 30 MDR-TB isolates retrieved from patients at the King George V Hospital, were determined using the Broth Dilution Method. To justify the perspective on cycloserine stability which follows, a brief description on the method that was used to prepare the cycloserine stock and results of the ongoing study follows: Aqueous solutions of cycloserine buffered to pH 10, were prepared on the day of the experiment using the method recommended by Sigma (2008). In general, a 100 × concentrated 64 µg/ml cycloserine stock solution was prepared by dissolving in 0.1 % sodium carbonate (Na_2CO_3) solution at pH 10. Subsequently, the cycloserine solution was filter-sterilized using a 0.22 µm sterile bell filter (Sarstedst, Numbrecht, Germany). The recommended method was used to alleviate cycloserine instability.

The results showed that the growth of 50 % of isolates were inhibited at 32 µg/ml, 40 % at 64 µg/ml, and 10 % at > 64 µg/ml of cycloserine. However, one isolate was classified as being atypical because on the day of reading the MIC result, distinct growth was observed in the wells containing 16 µg/ml and 64 µg/ml of cycloserine. We ruled out the possibility of this being due to inappropriate colony counts, as the colony forming units per millilitre (cfu/ml) of this isolate was related to other isolates having valid results. The sudden switch from being inhibited to having gained viability suggested that this isolate need be genotypically characterised, because the phylogenetics from being a susceptible TB isolate to MDR-TB isolate is not mediated by resistance plasmids, as is the case in other micro-organisms such as *Haemophilis ducrey*. Instead, MDR-TB arises from the selective pressure imposed on, and experience by, TB isolates by cycloserine (in this case), resulting in chromosomal mutations (Petrini and Hoffner, 1999). Therefore, this switch is not ascribed to the pre-determined pH condition because only plasmid-mediated insertion of resistant genes can occur spontaneously.

I (Rishan Singh) would now report a perspective on how the effects of pH on cycloserine is non-existent. The 7H9 broth that was used in BMM has a pH of 7.2, which was obtained using a strong base (NaOH). The chemical name of cycloserine is known as (4R)-4-Aminoisoxazolidin-3-one (WHO PAR Part 4, 2007). On the basis of this chemical formula, it is evident that the structural formula would incorporate 3 functional groups in its nomenclature. These functional groups are an amine, a ketone and an ether. According to the rules of chemistry, because the chemical formula starts with the 'amino' group, it takes on a lower priority compared to the ketone group 'one'.

When cycloserine is added to a solution of NaOH, the ketone group (C=0) changes to the H-C-O^- state, because NaOH has an electron-rich hydroxyl ion (OH^-), which induces this change. This expels the resultant cycloserine structure in solution as it is, since there is no leaving group. Since there is no leaving group, cycloserine is optimally stable in solution.

The optimal pH range for cycloserine is 5.0 – 7.4 (Official Monographs for Part 1). The previous study established optimal killing at a pH of 7.2, which is relatively neutral. Therefore, there are no electron-rich or deficient ions in broth. This implies the absence of leaving groups which would otherwise confer instable properties to cycloserine. It can therefore be implied that within the pH intervals of 6.8 – 7.2, cycloserine would be stable and that at pH's of <6.8 (electron-deficient ions) and >7.2 (electron-rich ions), the pH may cause cycloserine to become unstable. In summation, any instability caused to cycloserine in ranges 5.0 – 7.4, would therefore be ascribed to the broth (irrespective of the pH). This is in keeping with the generalisations by Martin-Casabona *et al*. (1997) and Victor *et al.* (1997) who have stated that drugs in different culture media become unstable (Martin-Casabona *et al*., 1997; Victor *et al*., 1997). This deduction has never been explained in TB research before and it is certain that it could form the basis of other studies involving unstable drugs in broth-based systems. I consider this perspective to be of critical importance to microbiologists and microbiological chemists who work in tuberculosis research and diagnostic laboratories throughout the work. In addition, the principle surrounding this deduction can be applied to studies involving other microorganisms and drugs.

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