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Commentary Open access

Thioridazine: A Therapeutic Panacea for Efflux Pump Mediated Multidrug Bacterial Infections

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Commentary

It is estimated that most of the medicinal compounds developed during the 20th century have their origins in phenothiazines [1]. Among these medicinal compounds is the first neuroleptic chlorpromazine (CPZ) introduced in 1957. The wide use of CPZ resulted in many published observations that indicated activity against a wide variety of microorganisms that cause therapeutically problematic infections [2]. CPZ has been shown to have in vitro activity against antibiotic susceptible [3] and resistant strains of Mycobacterium tuberculosis [4], has cured the aortis monkey of an antibiotic resistant malaria infection [5] and has yielded a temporary cure of prion promoted CJD [6]. Because CPZ is a very noxious compound that produces frequent serious negative side effects, there has always been resistance to use of CPZ for therapy of non-psychotic pathologies. The replacement of CPZ by thioridazine (TZ), a phenothiazine that is far milder than CPZ and as effective as CPZ for therapy of pyschosis, promoted studies that investigated whether similar antimicrobial activities produced by CPZ are also produced by TZ. This commentary provides a brief review of the studies that indicated that TZ has the potential to cure multi-drug resistant and extensively drug resistant tuberculosis and a wide gamut of multi-drug resistant bacterial infections whose phenotype is mediated by over-expressed efflux pumps.

Multi-Drug Resistant (MDR) Tuberculosis Infections

Tuberculosis infections continue to kill over 2 million people annually and at the time of this writing, infections that express an MDR and extensively drug resistant (XDR) phenotypes of Mycobacterium tuberculosis (Mtb) are therapeutically difficult to treat and extol high mortality [7]. TZ has significant in vitro activity against MDR TB [4] and XDR TB [8], enhances the killing of intracellular antibiotic susceptible [9], MDR drug resistant [9] and XDR Mtb strains [10], cures the mouse of antibiotic susceptible [11] and MDR TB infections [11,12], and cures XDR TB infected patients when used in combination with antibiotics to which initial resistance was present [13]. The use of TZ for therapy of latent tuberculosis seems promising given the effects of its killing of dormant Mtb [14,15]. The mechanism by which TZ cures a pulmonary TB infection involves a large number of targets depending upon the concentration of the agent. At low but effective in vitro concentrations it is a powerful inhibitor of efflux pumps that render the MDR TB phenotype resistant to two or more antibiotics [16,17] and at higher concentrations it inhibits the expression of genes that code for essential plasma membrane proteins [18]. Because of the multiplicity of targets, developed resistance to TZ is less likely than that taking place with conventional antibiotics. Moreover, it is quite safe when used for the therapy of XDR TB [13,19] and has been used as a salvage drug that rapidly improved the quality of life of the XDR TB patient [19].

As a consequence of the studies that examined the mechanism of its action against antibiotic resistant Mtb that has been phagocytosed by non-killing human macrophages [8-10,20,21], TZ may enhance killing of macrophage trapped Mtb by inhibiting K^+ and Ca^{++} efflux pumps of the macrophage that prevent the loss of these ions from the phagolysosome [20,21]. Inhibition of the loss of K^+ and Ca^{++} activates vacuolar hydrolases within the phagolysosome resulting in the degradation and killing of phagocytosed Mtb. These studies therefore suggest a mode of therapy that is a major departure from current practice; namely, that therapy targets the macrophage for activation of its killing machinery as opposed to targeting the phagolysosome trapped Mtb itself [22]. Moreover, the enhanced killing of entrapped Mtb bypasses any inherent limitation imposed by the degree of drug resistance of the organism. Because TZ is concentrated by lysosomes at least 100 times over that of the medium [23], its antimicrobial activity as well as its activity against the over-expressed efflux pumps that render Mtb with an mdr phenotype may contribute to the killing of the entrapped Mtb [16,17].

Activity against Gram-Positive and Gram-negative Bacteria

The activity of TZ on bacteria appears to be different for Gram-positives versus Gram-negatives. Firstly, whereas the minimum inhibitory concentration (MIC) of TZ against Gram-positives is about 15 mg/L [24,25], it is considerably higher for Gram-negatives and ranges from 60 mg/L for E. coli strains [26, 27] and 100 mg/l or greater for Salmonella [28]. However, with respect to Salmonella, the MIC assay does not tell the entire story of the interaction between the organism and TZ. Time-curve studies show that at the concentration of TZ that is just below the MIC, inhibition of growth is evident during the first 8 hours of culture. However, after this period, the organism increases its resistance to the agent such that by the end of 16 hours, it is resistant to a concentration that is equal to the MIC [28]. Evaluation of genes that code for the regulation of the acrAB-TolC efflux pump of the organism shows that during the first 8 hours of contact with TZ during which time the organism is not replicating, the sequence of gene activation first involves the sox gene, this is followed by the global regulator ram gene, and then followed by the local regulator mar gene. This cascade of sequential regulatory genes results in the activation of the genes that code for the transporter component of the main efflux pump of the organism acrB transporter [28] that eventually renders the organism resistant to very high concentrations of TZ. However, complete inhibition of growth can be maintained with a concentration of TZ that is below that which promotes any

inhibition of growth and an antibiotic substrate to which the organism is initially resistant. These results suggest that the inhibition of growth is facilitated by the inhibition of efflux of the antibiotic. If initial resistance to the antibiotic is due to a mutated target, a concentration of TZ below its MIC will not inhibit the growth of Salmonella. Unlike the situation with respect to TZ and Salmonella, the concentration of TZ at its MIC promotes complete inhibition of E. coli for the entire culture period [29]. It is probable that the difference between the two species with respect to the response to TZ lies in the presence of the global regulator ram of salmonella that is not present in E. coli [30].

The mechanism by which TZ inhibits the efflux pumps of gram-negative bacteria is indirect and involves denial of cellular energy that is dependent on a neutral pH of the periplasmic space. At pH above 6.5, metabolic energy is an absolute requirement for the maintenance of the proton motive force (PMF) that provides the energy for efflux pump function [31]. At a pH in the environment that is lower than 6.5, the mobility of hydronium ions from the surface of the cell into the periplasm maintains the PMF and insures the function of the efflux pump in the absence of metabolic energy [32]. At an environmental pH that is lower than 6.5, TZ has no effect on the activity of the efflux pump [32]. Hence, although not completely proven, the effect of TZ on efflux is indirect and involves denial of metabolic energy.

Because TZ affects the activity of efflux pumps, and secretions from the cell appear to be mediated by the main efflux pump of the bacterium, the secretions that promote quorum sensing (QS) activities [33,34] and secretions of biofilms [34] are inhibited by TZ. Because these efflux pump secretions complement the resistance mechanisms of the bacterium, the potential role of TZ as an anti-bacterial/inhibitor of efflux pump activity in combination with antibiotics is promising.

Conclusion

The study of the activity of TZ on a variety of bacteria that produce therapeutically problematic infections, all of which have been confirmed, strongly suggest that TZ has antibiotic potential for therapy of these infections. However, therapy with TZ should be administered with antibiotics to which initial resistance is due to an over-expressed efflux pump. That this suggestion has validity, TZ in combination with antibiotics to which the infecting XDR Mtb was initially resistant has yielded cures of XDR TB patients. Moreover, it has been used safely for the therapy of XDR TB and it is highly probable that when used in combination with antibiotics, it will be successful for therapy of other multi-drug resistant bacterial infections whose resistance is due to the over-expression of the main efflux pump of the infecting bacteria.

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