Antimicrobial Activity of Phenothiazines and Evolution of Anti-Tubercular agents from Non-Antibiotics: Hope against Multi Drug Resistant Tuberculosis

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Abstract

The antimicrobial action of a number of non-antibiotic drugs has been demonstrated in recent years such as the anti-tubercular activity associated with the Phenothiazine (PZN) neuroleptic class. However, as with other classes of non-antibiotics, such activity may be traced back to common structures like phenothiazinium dye, methylene blue. Multidrug resistant tuberculosis (MDR-TB) is the major global lethal infections accounting for over 4 million deaths per year. Several of the lead compounds and their non-antibiotic derivatives may have a part to play in the treatment of infectious disease, mainly in the treatment of tuberculosis. This review provides the basis that supports the use of one particular group of compounds, PZNs and in particular Thioridazine (TZ), for the treatment of the antibiotic-resistant infections. Because TZ is a mild neuroleptic as compared to its parental compound chlorpromazine, kills intracellular MDR-TB at clinical concentrations, its use for the treatment of these infections may be considered.

Keywords: Antimicrobial Activity; Antibiotic Resistance; Neuroleptics; Phenothiazines

Introduction

Among therapeutic approaches, the use of antibacterial-natural product antibiotics or synthetic-/semi-synthetic agents-is widespread, the huge strides made against infection are no longer possible, mainly due to the widespread evolution of drug resistance mechanisms among bacteria, in some cases conferring resistance to all known clinical agents. A prima facie example of this phenomenon is that of Mycobacterium tuberculosis (Mtbc), the principal causative agent in pulmonary Tuberculosis (TB) that has evolved from Multi-Drug Resistant (MDR, resistance to Isoniazid (INH) and Rifampicin (Rif)) to Extensively Drug Resistant (XDR, resistance to INH, Rif, streptomycin, any fluoroquinolone and any of the inject able anti-TB drugs amikacin, kanamycin and capreomycin) and more recently to Total Drug Resistance (TDR) [1]. In each case, whether MDR/XDR/TDR TB is due to over- or mis-use of the available agents, or required prolonged therapy involving periods of many months-or even an excess of one year as is the situation for therapy of a new case of TB-or simply because of therapeutic longevity, sufficient selective pressure has been brought to bear, and alternative drugs are in very short supply indeed. This situation is not helped by the single mode-of-action antibacterial approach utilised in the majority of cases. This provides effective selective pressure, it being relatively simple to nullify such drugs in the laboratory via single-point mutation to one member of a drug class, and this may be readily followed by resistance to two or more drugs [2]. Inducement of MDR may involve resistance to structurally-similar drugs due to the mutated target [3], or to drugs that are not structurally related [4]. Clearly there is an argument here for the introduction of less-targeted therapeutics, at least in terms of microbial anatomy. These are currently rare in practice, and multi-site attack relies on therapeutic co-administration. Nor is the parlous state of our antimicrobials the sole concern. Damage to the internal flora following a period of oral use is often also a cause of illness, and is particularly problematic in the elderly due to the survival and overgrowth of refractory organisms, such as the Gram-positive anaerobe Clostridium difficile or the yeast Candida albicans [5].
The term chemotherapy was coined by the German scientist Paul Ehrlich. The ideas on differential cell staining by aniline dyes during the early 20th centuries laid the foundations for what became known as selective toxicity, a principle which still underpins our modern use of antimicrobial drugs. However, Ehrlich’s principal contribution to drug discovery was to realise that there was a relationship between dye chemical structure and cell, or organelle, specificity. This quantum leap was manifest in his involvement in the successful clinical treatment of malaria [6], for which Ehrlich used the phenothiazinium dye methylene blue. Globally, the emergence of MDR-TB is an increasing problem that affects public health and patient care. It is estimated that 1.7 to 2.0 billion humans are infected with Mt [7]. An estimated 1.5 million people died from TB in 2006 [8]. India has more new TB cases annually than any other country, ranking first among the 22 high burden TB countries [10]. TB remains one of the leading infectious causes of mortality in India, resulting in 364,000 deaths annually. There were more than 1.8 million new TB cases in India in 2004, representing over one-fifth of all TB cases worldwide. The estimated incidence rate in 2004 was 168 per 100,000 people [10]. There are estimated 450,000 new cases of MDR-TB around the world every year [11,12]. Emerging anti-TB drug resistance in India deserves serious attention as India’s rate is highest among 22 high-burden countries [10].

Classification of Drug resistant TB

- **Poly drug resistant Tuberculosis**: Resistance to two or more antibiotics [7].
- **Multidrug Resistance Tuberculosis** (MDR): Resistance to at least rifampicin and isoniazid [7].
- **Extensively Drug Resistant Tuberculosis** (XDR): resistance to at least INH and Rifampicin (i.e. MDR-TB), a fluoroquinolone and to one or more of injectable amino glycosides (amikacin, capreomycin, kanamycin) [10]. In cases of MDR-TB the main line of treatment is the 2nd line drugs. The problems in countries like India are:
  1. Lack of laboratory procedures required for the delivery of antibiotic susceptibility data results in blind treatment of infection.
  2. The 2nd line drugs are expensive and duration of treatment is considerably longer.

These factors further lead to an increase in the incidence of MDR-TB. The solution lies in providing an alternative that has the potential to treat patients effectively regardless of the antibiotic susceptibility profile of the causative organism at a cost that is affordable by the most economically deprived country.

- **Phenoethiazines** (PZNs) are a class of drugs that have significant *in vitro* activity against susceptible, polydrug- and MDR-TB, as well as enhancing the activity of some agents used for first-line treatment. The PZNs have the potential as an adjunct to conventional therapy during the lengthy period before antibiotic susceptibility data are available. Moreover they may reduce morbidity linked with the use of rifampicin and streptomycin by allowing their use in lower dosages. The need for new and effective anti-TB drugs is recognized, and PZNs: hope against MDR-TB it is this need which has spurred a renewed interest in pursuing the question of whether another less toxic PZN may be exploited as an anti-TB agent. The present article describes the role of PZNs as an adjunct therapy in anti-TB treatment and their role in MDR-TB.

History

The antimicrobial activity of Phenoethiazines (PZN) has been known since the time of Paul Ehrlich [13]. However, because methylene blue had been shown to have neuroleptic properties, its antimicrobial properties remained essentially underscored and, instead, derivatives of methylene blue were eventually synthesized and used effectively for the therapy of psychosis. The first such compound, Chlorpromazine (CPZ) and because of its wide and extensive use, its antimicrobial properties were soon evident. However, because the golden age of antibiotics began at this time, there was no need for CPZ, or indeed any of its derivatives, to be considered as antimicrobial agents. Furthermore, because prolonged use of CPZ produced a number of serious side-effects [14], and whatever antimicrobial activity reported was essentially one that was produced *in vitro* and at clinically irrelevant concentrations [15], CPZ or other PZNs were not seriously considered as potential sources of new antibiotics, even when they were shown to have desired antimicrobial effects *in vivo* [16]. However, the global increase of MDR-TB, quinidine-resistant malaria, nosocomial MRSA infections, *etc.*, primarily in countries that cannot afford available antibiotics, not with standing the problem of resistance, dictates that PZNs be now considered where other drugs have failed.

The Phenoethiazine (PZN) neuroleptics were developed as a result of clinical observations arising from the use of methylene blue in psychiatry. A series of PZNs were tested, those with the alkylnino-alkylamino-side chain (including the ring nitrogen) often exhibiting activity, usually either as H1 or D2 antagonists, depending on the length of the side chain, e.g. Promethazine and Chlorpromazine (CPZ) respectively. The CPZ became a widely used major tranquiliser for use in the treatment of schizophrenia, and improved derivatives, such as Thoridizine (TZ). In addition, bioisosteric replacement of N-10 in the PZN chromophore with trigonal carbon furnished the thioxanthenes, such as flupenthixol, and the alkylnino-alkylamino-side chain can still be seen in more modern D2/5-HT-active agents such as clozapine and olanzapine. Similarly, analogue based on the PZNs led to the development of the tricyclic antidepressant class, including imipramine and amitriptyline.

Figure 1: Drug evolution from methylene blue (phenothiazinium class), significant alterations: a= side chain variation; b = chromophore change.

The combination of a basic side chain and a tricyclic–normally heterocyclic-chromophore may be observed in each of these therapeutics. Functionalised methylene blue derivatives are potential antimicrobial agents (Paludenblau and modern CNS agents). While these chemists were not working to a molecular targeting paradigm, as would be expected in modern pharmaceutical research, the compound screening protocols normally included small animals. Methylene blue is a highly hydrophilic dye. This is reflected in its short half-life in mammalian systems, the greater proportion of the compound appearing rapidly in the urine. It has been proposed that the uptake of methylene blue by microbes involves this reduction stage, which facilitates its passage to the cell interior where it may be re-oxidised in the cytoplasm. Methylene blue in a small animal TB model: grey-coloured tubercles in the post-mortem lung turned blue on exposure to the air.

CNS agents

The early Phenothiazine (PZN) neuroleptic drugs were developed rather empirically—not surprising, given the scant contemporary knowledge of receptor function. The removal of violent symptoms in schizophrenic patients, sufficient for their daily treatment, was a most persuasive argument for the clinical acceptance of chemical administration. However, the subsequent appearance of serious side-effects was an obvious indicator of a lack of correct selectivity [15,17]. The development of both the drug chromophore and side chain to this end obviously produced a considerable number of active compounds, covering a wide structural range. As mentioned, the spin-off development of the tricyclic antidepressants may be included here [18]. The whole collection thus represents a significant number of clinically-acceptable examples of the tricyclic chromophore / basic side chain molecular type.

Although the antimalarial [19] and antibacterial properties of CPZ have been known for decades, due to its frequent serious side effects, it has never been seriously considered for therapy of bacterial infections [13,15,20-22]. However, with the advent of MDR TB infections in the 1980s in areas where TB was thought to have been reduced to the point of eradication, the search for effective anti-MDR TB compounds was begun. Among the compounds examined was Largactil, then the commercial preparation of CPZ, which had been shown in the 1950s to cure TB infections and later to have in vitro activity against all forms of antibiotic-resistant strains of Mtb. However, the in vitro activities occurred at concentrations which were beyond those clinically achievable in serum, but CPZ and other PZNs have very high affinity for the lungs, ≤ 100 μg/g wet tissue. At this concentration found that all mycobacteria, independent of resistance pattern, were inhibited by ≤ 25 μg/ml of CPZ on agar. Although this concentration is hundreds of times greater than that safely possible in the human, PZNs are known to be concentrated by up to 300% in the tissues, allowing the possibility that the antibacterial concentration might be reached in vivo.
These results were confirmed in 1992 and it was also demonstrated that CPZ kills Mtb in newly-phagocytosed human macrophages [23,24], but interest in CPZ remained low—the CNS side-effects of the agent prevented any serious consideration of its use as an anti-TB drug. Nevertheless, since Thoridazine (TZ), the neuroleptic that replaced CPZ, is a much milder drug producing fewer serious side effects than CPZ and was the in vitro equal of CPZ with respect to its activity against all forms of antibiotic-resistant strains of Mtb, interest in TZ as an anti-MDR TB drug gained credence and it was soon shown to promote the killing of multi-drug resistant strains of Mtb by non-killing macrophages at concentrations in the experimental medium that were below those used in the initial therapy of psychosis [23,24]. TZ has now been shown to cure both antibiotic-susceptible and MDR TB infection in a murine model, and ten out of twelve cases of XDR TB patients. Protocols have now been published for the therapy of XDR TB whose prognosis is solemn, and may be conducted on a compassionate basis mfs [25-28].

The widespread use of Chlorpromazine (CPZ) during the 1950s also yielded anecdotal observations that the PZN acted synergistically with anticancer therapy. This synergism was later shown to result from the inhibition of the eukaryotic transporter P-glycoprotein (Pgp1) which when over-expressed renders the cancer cell susceptible to the anticancer agent to which it was originally resistant [29]. Similarly, the demonstration that CPZ reduces sensitive and resistant bacteria strains to antibiotics [30,31] was later interpreted to result from an inhibition of an efflux pump system that extruded the antibiotic prior to its reaching its intended target [32]. Since these studies, PZNs have been shown to inhibit the NorA efflux pump of Staphylococcus aureus [33], the QAC efflux pump of the plasmid carried by a S. aureus MDR strain [34], the efflux pump system of E. coli [35], the AcrAB efflux pump of E. coli [2,36], the main efflux pump of M. smegmatis [37] and the efflux pump of M. avium [38]. The PZNs CPZ [39] and TZ [40] have been shown to affect the activity of genes that regulate and code for the AcrAB efflux pump of E. coli as well as that of Salmonella enteric serovar Enteritidis [41]. That the activity of TZ on bacterial genes is probably universal is illustrated by its effects on the main survival genes of Mtb [42].

Although nothing has essentially been reported with respect to Structural Activity Relationship (SAR) for TZ derivatives that are effective against Mtb, a recent study focused on synthesized quaternized CPZ, triflupromazine, and Promethazine derivatives, demonstrated that these derivatives had anti-TB activities against both actively growing and non-replicating references wild-type Mtb H37Rv [46]. All active compounds were found to have no toxicity against a Vero cell line. Importantly, whereas N-Allyl-chlorpro-mazinium bromide rendered the derivatives less active, the replacement of the allyl with benzyl or substituted benzyl promoted significant greater anti-TB activity. Moreover, the substitutions that had powerful electron withdrawing properties were essential for the improved activity of the derivatives. All derivatives that contained branching at the carbon chain expressed significantly weaker activity. The main conclusion that can be drawn from these studies is that structures that possessed N-(4- or 3-chlorobenzyl) substitution expressed the highest anti-TB activity.

The PZNs appear to have other antibacterial effects, causing the elimination of plasmids from Gram-negative [47,48] and Gram-positive bacteria [49,50]. The PZNs promote the elimination of plasmids from bacteria (plasmid curing) due to the smaller concentration (MIC) of the agent needed to inhibit plasmid replication as opposed to those required for the inhibition of replication of the bacterium. This inhibition results from the intercalation of the agent between nucleic bases of DNA, especially at regions rich in guanosine and cytosine [51]. However, because PZNs have significant effects on the cell envelope of bacteria [52,14] the more facilitated elimination of smaller plasmids as opposed to larger plasmids may be, at least partially due to the size of the plasmid itself [53]. Because the antibiotic resistance of bacterial pathogens relevant to human and animal husbandry is often due to plasmids carrying antibiotic resistance genes [54], the elimination of plasmids from bacteria provides a way to eliminate clinical antibiotic resistance in infection-causing bacteria. Interestingly, because industry has been told that processes employing bacteria must be conducted with bacteria that have no antibiotic resistance, elimination of plasmids is now being pursued vigorously [55].

During recent years pre-administration of PZNs to animals has provided protection from virulent Salmonella infections [56,57]. Because protection takes place with in vivo concentrations of the PZN which are 30 times lower than those needed to inhibit the in vitro replication of the organism, the mechanism by which protection is afforded is not dependent upon the effects of the agent noted in vitro. If the in vivo concentration of the agent is so low, how does it protect from an infection by a highly virulent Salmonella strain? Moreover, because the mode of infection involved IP administration and subsequent rapid phagocytosis of the bacteria by neutrophils does not result in the killing of the organism, how does the agent protect from infection? The answer may lie in the following: within minutes of phagocytosis and fusion of the
lysosome with the phagosome, the low pH of this vacuole induces the activation of two two-component regulons; PmrA/B and PhoP/Q. The activation of these regulons results in the rapid synthesis of Lipid A which is introduced into the nascent lipopolysaccharide layer of the outer cell wall. Once this occurs the organism is resistant not only to the hydrolytic enzymes of the phagosome-lysosome vacuole, but also to practically everything else [58] (Gunn. 2008). It is postulated that because pre-use of the PZN results in the accumulation of the agent by lysosome-rich macrophages such as the neutrophil [59,60], the concentration of the PZN within the lysosome may be sufficiently high to inhibit the first step of the two-component regulon-namely, the sensor function of the PmrA receptor present on the surface of *Salmonella*. Support for this possibility is provided by the non-specific binding of the PZN CPZ to the surface of the cell blocking access of an O antibody to the O antigen surface of the bacterium [54]. Nevertheless, other possibilities that contribute to the protection provided by the PZN exist in as much as the PZN CPZ has been shown to modulate secretion and syntheses of cytokines involved in protection from infection [61].

**The lipophilic chromophore/basic side-chain paradigm**

In Denmark membrane stabilisers such as anaesthetics, the Phenothiazines (PZNs) and chloroquine have been studied in respect to efflux inhibition, and investigated the inhibition of potassium efflux and antimicrobial, immunostimulation and reversal of resistance potency in the same compounds [62]. Interestingly, the observed antimicrobial activities of different PZN and thioxanthene analogues and their metabolites [63] are independent of their CNS activity as seen, for example, in the antihistaminic and neuroleptic PZNs. It can be stressed that PZN analogues with an exocyclic double bond (the thioxanthenes) are more potent antimicrobials *in vitro* than the corresponding compounds lacking it. The (Z) isomeric thioxanthenes (Figure 2) generally, exhibit stronger neuroleptic activities compared to the (E) isomers. The possibility therefore exists to separate the CNS and antimicrobial activities by choosing the latter compounds as antimicrobials and increasing this activity by optimising ring substitution using e.g. halogens [63].

**Figure 2**: The alkyl amino-alkyl amino-type side chain shown in Paludenblau, and emphasised in subsequent neuroleptic molecules.
Similar behaviour is exhibited by classical PZN compounds exhibiting (+/-) stereochemistry, e.g. Promethazine, alimemazine, mepromazine and TZ [64]. (+/-)TZ is especially important, because this mixture exhibits very potent antimicrobial activity against both resistant (MDR, XDR) and sensitive mycobacterial strains. These compounds are also very active as efflux inhibitors in the reversal of antimicrobial resistance. Unexpectedly, (-)-TZ, without CNS activity, is the most potent [64]. This observation constitutes a medical breakthrough for the use of PZNs and their analogues, as well as their more active metabolites, together with classical chemotherapeutics for synergy and/or for reversal of resistance in vivo. It is possible to use TZ as a “Helper compound” in MDR and XMDR TB investigations.

Similarly, successful treatment of seriously ill patients with XDR TB with low doses of TZ as a helper compound in a regime together with anti-TB compounds against which the TB strains are resistant [65]. Ehrlich had noted such possibilities in neurotropic compounds, in relation to his theories for developing chemotherapeutics from dye series to be used in serious intracellular infectious disease. Now, a century after Ehrlich, a PZN derivative synthesized in the late 1950s has a role to play in one of the most serious bacterial threats to mankind, XDR TB. The PZNs may now have a renaissance as a new antimicrobial class. The PZNs, thioxanthenes and dibenzazepine antidepressants, all obey the lipophilic chromophore/basic side chain paradigm. These lipophilic chemicals and their stereochemical variations have been investigated since late in the 1970s. However, so far there has been much less investigation of the modern antidepressant (SSRI) drugs as efflux inhibitors [33,66-69] than of the PZNs and other, older psychopharmacological agents.

It is perhaps ironic, then, that these less related CNS agents, such as the selective serotonin reuptake inhibitors femoxetine, paroxetine, fluoxetine, sertraline and cipramil and their stereochemical analogues have now been shown to act also as antibacterial, antifungal, antiviral and antiprotozoal compounds both in vitro and in vivo and as efflux pump inhibitors, exhibiting particular activity against different Gram-positive and Gram-negative bacteria [33,66-69]. However, on structural consideration, each of these SSRIs can be seen to fulfill the same chromophore/side chain requirement (Figure 3), and so might also be used as helper compounds in the future as demonstrated by Cecchelli et al. in 2010 in the treatment of HIV infected persons [70].

Phenothiazines as Anti-TB agents

The Phenothiazine (PZN) groups of drugs are tricyclic compounds that are used as antipsychotic agents. They were once the most widely used antipsychotics. They are Aliphatic derivatives: Chlorpromazine (CPZ) and Thioridazine (TZ); and Piperazine derivatives: Trifluperazine, Perphenazine and Fluphenazine. Piperazine derivatives are more potent as they are effective in lower doses [71]. Methylene blue, the first PZN, was an aniline dye. Ehrlich had demonstrated that it had activity against Plasmodium falciparum, and when administered to patients would cause them to become lethargic. The wide acceptance and use of CPZ in the ensuing years for the treatment of severe neuroses and psychoses yielded a few anecdotal reports suggesting that this agent had anti-TB properties. Interest in the development of these compounds as anti-mycobacterial agents did not materialize because of the by then well-known severe side effects produced by the chronic administration of CPZ. The introduction of isoniazid in the 1950s for the treatment of infections by Mtb, later followed by other effective compounds (streptomycin and rifampicin), lessened any further interest in the use of CPZ as an anti-TB drug [72].

Inspite of the fact that PZNs are active against Mtb they were never considered seriously as ATT because the lowest concentration required for significant in vitro inhibition of growth greatly exceeded that achievable in patients (i.e. about 0.5 mg/L) receiving a minimum dose of 600 mg/day and severe side effects that are associated with its chronic use [73]. However, in 1992, Crowle et al demonstrated that CPZ could inhibit the growth of Mtb that had been phagocytosed by human macrophages when CPZ was present in the medium at concentrations ranging from
0.23 to 3.6 mg/liter. Mtb phagocytosed within the macrophages was susceptible to concentrations of CPZ 10 times lower in the culture medium than those needed for a similar inhibition of unphagocytosed bacteria. These concentrations were within the range anticipated in patients treated with this PZN. The macrophages had the ability to concentrate the PZN, an interpretation consistent with those studies showing that CPZ was found in pulmonary tissue in concentrations in excess of 100 times those in plasma.18 in early 1990s the problem of TB was not significant in most Western countries. Therefore, CPZ, which produces serious side effects, was not considered for use in the treatment of TB. With the worldwide resurgence of TB and increase in MDR -TB, there is an urgent need of newer agents and hence the renewed interest in PZNs as Anti-TB agents. The CPZ, the first commercially produced PZN for the treatment of psychosis, was also one of the first of the PZN series shown to have anti-TB properties [23,74,75]. The MIC of various PZNs is shown in Table 1. Of all the PZNs Trifluperazine appears to have most potent Anti-TB action.

<table>
<thead>
<tr>
<th>Phenothiazine</th>
<th>MIC(mg/L)</th>
<th>System</th>
<th>Phenothiazine</th>
<th>MIC(mg/L)</th>
<th>System</th>
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<tbody>
<tr>
<td>Chlorpromazine</td>
<td>12 Cfu</td>
<td>12 Cfu</td>
<td>Promethazine</td>
<td>20 Cfu</td>
<td>20 Cfu</td>
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<tr>
<td>10 Cfu</td>
<td>10 Cfu</td>
<td>12.5 Cfu</td>
<td>12.5 Cfu</td>
<td>&gt;25 Bactec</td>
<td>&gt;25 Bactec</td>
</tr>
<tr>
<td>10 32Pi</td>
<td>10 32Pi</td>
<td>10 32Pi</td>
<td>Trifluoperazine</td>
<td>5-8 Cfu</td>
<td>5-8 Cfu</td>
</tr>
<tr>
<td>0.9 Mac</td>
<td>0.9 Mac</td>
<td>0.9 Mac</td>
<td>Methdilazine</td>
<td>5-15 Cfu</td>
<td>5-15 Cfu</td>
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<tr>
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<td>4-32 Bactec</td>
<td>Thoridazine</td>
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<td>8-32 Bactec</td>
</tr>
<tr>
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<td>6-12 Bactec</td>
<td>6-12 Bactec</td>
<td>Desipramine</td>
<td>&gt;25 Bactec</td>
<td>&gt;25 Bactec</td>
</tr>
<tr>
<td>Levomepromazine</td>
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<td>10 Cfu</td>
<td>4-32 Bactec</td>
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Table 1: Minimum Inhibitory Concentration of Various Phenothiazines in vitro.

The lowest effective in vitro concentration against Mtb is seen after the organism has been phagocytosed by human macrophages. In the absence of macrophages, the MIC is almost 10-fold higher, as a consequence of the ability of macrophages to concentrate CPZ, many times over that achieved in the medium.

**Mechanism of Action**

The calmodulin like protein has been demonstrated in mycobacterium and it has been observed that there is a positive correlation between the levels of CAMLP, phospholipids as well as lipids and growth. The PZNs are Calmodulin antagonists and therefore inhibit the growth of Mtb [76].

**Role of phenothiazines in MDR-TB**

It has been observed that all strains of Mtb tested so far, regardless of whether they are susceptible to all agents, or are multi- or polydrug resistant, or even resistant to all five primary antibiotics, are equally affected by CPZ [23,74,75]. Table 2 shows the effect of CPZ and other PZNs on various strains of Mtb. It is clear that the PZNs are equally effective against drug resistant strains as they are against the susceptible strains. This is of clinical importance in treatment of MDR TB. Furthermore it has been observed that CPZ increases the Anti-TB activity of Rifampicin and Streptomycin, thus permitting to give them in lower doses without sacrificing the integrity of treatment. The CPZ, TZ and Trifluperazine were shown to enhance the activity of Rifampicin and Streptomycin when used in combination at concentrations that are minimally effective when employed separately against clinical strains of polydrug resistant Mtb [13,77-79]. They however have no augmenting activity against ethambutol in any strain and INH in polydrug resistant strains.

**Choice among Phenothiazines**

The extensive use of CPZ for the treatment of psychosis during the past 40 years provided abundant evidence for this compound producing severe side effects when administered over long periods [80,81]. More serious side effects include: Cholestatic disease, acute liver injury, keratopathies, conjunctivitis, phototoxicity, photoallergy (dermal) and agranulocytosis.

These severe side effects associated with the chronic use of CPZ makes it suitable for use in the treatment of TB. Thoridazine (TZ) on the other hand is a compound with fewer side effects. It may cause transient mild retinopathy. Other than that the most common side effect is drowsiness. In experimental mice, chronic use of TZ has resulted in cardio toxicity. The same effect has not been noticed in humans except in cases of overdose or co administration with other potentially cardio toxic drugs [82-84]. Regardless of this, a careful monitoring of cardiac function during TZ treatment is strongly recommended. The use of TZ as the CPZ of choice for the treatment of freshly diagnosed pulmonary
TB is further reinforced by the observations that this compound enhances the in vitro activity of rifampicin and streptomycin against polydrug-resistant strains of Mtb. The use of PZNs for treatment of an active pulmonary TB infection caused by antibiotic-susceptible Mtb strains provides no advantage over the use of present therapies. The concentration of PZNs needed to kill or even inhibit mycobacterial replication when the bacterium is outside the macrophage is far beyond that which can be achieved in the patient so it cannot be used for the treatment of a cavitary pulmonary Mtb infection of moderate to severe status.

### Antimicrobial Activity of Phenothiazines

The antibacterial properties of Phenothiazines (PZNs) may be summarised as follows: gram-positive cocci [61,32] Mycobacteria [85,24] and some gram-negative rods, such as *Shigella* spp., are more susceptible to a number of PZNs as opposed to gram-negative rods such as *Escherichia coli* [60] and *Salmonella* spp. [15] in general, with MIC’s that range from 20 to 30 µg/ml for the “Susceptible group”, and in excess of 100 µg/ml for the “Resistant group”. It is important to note that, regardless of the method employed for assaying the activity of the PZN, all of the activities take place at concentrations that greatly exceed the highest plasma concentration achievable, namely 0.5 µg/ml [61]. Although this data at face value suggests that the antibacterial use of these compounds is not feasible, smaller concentrations of PZNs do enhance the activity of antibiotics to which the bacterium is susceptible [30,79,86], even when it is resistant to the antibiotic [32].

The latter observations suggest that these compounds may serve as adjuvant whenever there is a need to reduce the dose of a given antibiotic or render an antibiotic-resistant infection susceptible to the antibiotic. Nevertheless, although the concentrations of the PZNs that enhance antibiotic activity are significantly lower than those that have in vitro antibacterial effects, they are, in many cases, beyond that which is clinically relevant. The potential use of PZNs as antibacterial agents or as enhancers of antibiotic activity lies in their ability to kill phagocytosed bacteria. *Mtb* and *Staphylococcus aureus*, that have been phagocytosed by macrophages that by themselves have little killing activity of their own, are effectively killed [72]. Killing takes place at concentrations in the medium that are well within clinical levels and well below any toxic effects against macrophages or other cellular components of immunity [24]. Killing apparently is the result of the ability of the macrophage to concentrate the PZN to a level comparable to a minimal bactericidal concentration, a property previously shown for tissues and organs that are rich in macrophages [87,88].

For all studies to date, the effectiveness of Thioridazine (TZ), whether in vitro directly or as an enhancer of antibiotic activity, or for the reversal of antibiotic resistance (i.e. macrophage), is equal to that of the far more toxic CPZ. Because TZ kills intracellular MDRTB, it has the potential of being employed for the therapy of an individual who has recently sero-converted to a positive PPD and who resides in an area that is known to have a high frequency of MDRTB. TZ will probably prove to be ineffective therapy for patients presenting with cavitary disease exceeding that of moderate status, since the concentrations of TZ needed for killing extracellular MDRTB are well beyond which is clinically achievable. With respect to MRSA infections, TZ might be valuable for treating recurrent MRSA-vancomycin-resistant infections present in hyper-IgE syndrome [89] in febrile neutropenia accompanying cancer chemotherapy [90], and other diseases presenting with neutropenia and weak granulocyte functions, i.e., glycogen storage disease type Ib [91], whose basis for recurrence lies in the intracellular location of the organism that is not killed by the macrophag cell. The PZNs are known to alter the morphology of bacteria when the concentration of the PZNs is below that which inhibits the cell’s replication.

The alterations are specifically related to the species, such that the PZNs causes filamentation of *E. coli* [52] and *Salmonella typhimurium* [14] and cluster formation of *S. aureus* resulting from unseparated daughter cells. It is interesting to note that these respective responses to the PZNs are very similar to those evoked by sub-inhibitory concentrations of a beta-lactam. Because beta-lactams specifically bind PBP3 of a gram-negative bacterium such as *E. coli*, and such binding is associated with the filamentation of the bacterium, PZNs may either bind directly to a PBP or have some effect on other mechanisms that affect the PBP and subsequent filamentation ensues. Because filamentation of gram-negative bacteria can be produced by non-beta-lactam antibiotics such as quinolones, as well as by physical conditions such as release from hydrostatic pressure [92] and by growth conditions [93], the

### Table 2: Activity of CPZ, TRZ and TFZ against various strains of Mtb.

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Chlorpromazine</th>
<th>Thioridazine</th>
<th>Trifluperazine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of isolates</td>
<td>Lowest effective Conc</td>
<td>No of isolates</td>
</tr>
<tr>
<td>None</td>
<td>22</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Multidrug resistance</td>
<td>21</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Polydrug resistance</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>INH,RIF,STR,EMB</td>
<td>11</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

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filamentation of a gram-negative rod caused by sub-inhibitory concentrations of a PZN may not involve a direct effect on the PBP itself. The PZNs have been shown to reduce the adherence of gram-negative bacteria to epithelial cells. The PZN Promethazine prevents the recurrence of pylonephritis caused by E. coli in pediatric subjects [50] and, because the concentration of this PZN required to inhibit the growth of bacteria is well beyond that clinically relevant, the successful therapy of recurrent pylonephritis is probably due to the effect the PZN has on the adherence of E. coli to the epithelium of the urinary bladder, the latter being a prerequisite for eventual development of pylonephritis. Although the effects of a PZN on structures of the gram-negative bacteria needed for adherence such as pili, or its effects on molecules present on the surface of epithelial cells that are to a lesser extent required for the adherence of the bacterium, have not yet been fully studied, it seems probable that PZNs do inhibit adherence by inhibiting pili formation, much as is true with low concentrations of antibiotics [94], as well as by interfering with access by bacterial pili to receptors present on the surface of the epithelial cell.

The in vitro and ex vivo antibacterial activities of PZNs described most probably account for cures of bacterial infections treated with PZNs. Mice infected with Salmonella typhimurium can be cured with Trifluparazine [95] or Fluphenazine [57], by a combination of trimethoprim and trimethoprin [96]. Pre-treatment with 10-[n-(phthalimido) alkyl]-2-substituted-10H-phenothiazines or 1-(2-chloroethyl)-3-(2-substituted-10H-phenothiazines-10-yl) alkyl-1- ureas protected the animals from lethal infection of E. coli to various extents; and mice infected with Mycobacteria could be cured with methdilazine [77]. The curative effects of CPZ on humans presenting with bacterial infections are also known and have been reviewed elsewhere. Although there currently exists sufficient support for the use of PZNs, especially the far less toxic TZ, for the therapy of problematic infections caused by antibiotic-resistant bacteria, their use is not recommended at this time unless there is a need for compassionate therapy, i.e., nothing else is available.

Antimalarial Activity

The antimalarial activity of Phenothiazines (PZNs) has been known for over a century [15]. However, because chloroquine has been so effective for the major part of this period, there was no need for another antimalarial. This situation has, of course, changed given the global advent of increasing antimalarial-resistant infections [97]. Because there are no effective antimalarial drugs other than chloroquine available to indigenous people who reside in areas of the world where malaria is still the major lethal infection [98], there is a dire need for effective antimalarials. CPZ and other PZNs are known to have in vitro activity against Plasmodium falciparum [99-101]. The CPZ effectively cures the Aortus monkey of a P. falciparum infection and reverses resistance to chloroquine. However, since not all Plasmodia resistant to chloroquine can be made sensitive to the antimalarial [99], this may indicate strain-and/or species-based differences with respect to the modulation of antimalarial resistance by the PZN. Although no study to date has tested the effectiveness of TZ as an antimalarial, it is highly probable that it, too, may be as effective as CPZ [15].

Antiprotozoan Activity

Leishmaniasis is an infection caused by protozoa belonging to the genus Leishmania. The disease, expressed in humans as cutaneous, visceral and mucocutaneous leishmaniasis, has a wide epidemiological range; globally, it infects more than 300 million people and accounts for approximately 1 million deaths per year [102]. Therapy for this infection is problematic since the side-effects produced by conventional drugs are considerable [102] and, as is the case with bacterial and malarial infections, resistance to drugs such as antimonials is quite common today [103]. The PZNs and acridines have long been known to have activity against Leishmaniasis-causing parasites, however, the concentrations needed for this activity are either toxic or clinically irrelevant. Although topical application of CPZ has been reported to effectively cure cutaneous leishmania, others claim otherwise. Nevertheless, because Leishmania is an intracellular parasite, CPZ will kill the intracellular organism. TZ will probably prove to be as effective against intracellular species of Leishmania.

Antiviral Activity

The CPZ has been shown to have activity against a broad gamut of viruses. As early as 1971, it was shown to inhibit the modification of host cell membranes caused by herpes simplex. Thereafter, it was shown to inhibit the growth of TBEV, inhibit the activity of hepatitis B DNA, lyse a number of viruses, inhibit the conjugal transfer of R and F’lac plasmids, inhibit the budding of measles virus and Sindbis and vesicular stomatatis virus, inhibit the replication of influenza virus, SV40 [104], arenavirus [105] and HIV [106], block infection of B lymphocytes by human herpes virus [30] and infection of tissue culture cells by JC virus (JCV) [107]. The mechanisms by which CPZ produces the effects noted may be grouped as follows: it inhibits binding of virus to receptor of the plasma membrane [108], it inhibits calcium-dependent events that take place at the plasma membrane and which are required for entry of the virus via endocytosis [109], and it inhibits the replication of DNA primarily by intercalating between the bases [110].

Although all of the effects of CPZ on the virus itself or the plasma membrane of its target cell take place at concentrations which are clinically irrelevant, the drug has served as a “lead compound” for the synthesis of a variety of derivatives which have similar activities at significantly lower concentrations. Moreover, the antiviral activity of the PZN methylene blue can be substantially enhanced when the presentation of this compound to a virus takes place under photo-activation [111]. The enhanced antiviral activity
of methylene blue by photo-activation has been known for over 7 decades and has been only recently employed successfully for deactivating virus present in blood transfusion products such as whole blood [112], plasma, platelet concentrates and coagulation factors [113,114], and cryoprecipitates and cryosupernatants [115]. The successful use of photo-activated methylene blue has prompted consideration that this approach may have some value for managing problematic viral infections.

Antiprion Activity

The CPZ and acridines have eliminate the presence of prions of infected mouse neuroblastoma cells chronically infected with the prion PrP(Sc) [116]. These results were effective but temporary therapy of two young women presenting with CJD. The theoretical mechanism which PZNs destroy intracellular prions and the suggested treatment of the prion infections [117].

Plasmid Elimination/Curing Effects of Phenothiazines

Resistance of a given bacterial species to one or more antibiotics may be acquired in the host by the transfer of mobile genetic determinants such as plasmids and transposons from another unrelated species [118,119]. Antibiotic-resistant genes present in plasmid-containing bacteria can cause serious therapeutic failure [120,121] or manifest as a consequence of the selection of the resistant plasmid-containing strain. With these facts in mind, compounds that can neutralise the potential effects of plasmid antibiotic-resistant genes in a given bacterial infection are clinically important. To this extent, PZNs are known to promote the elimination of plasmids from infected bacteria [122,50].

Future Considerations

Because of the urgency required for the therapy of an essentially lethal infection, this review primarily focused on the potential that the PZN, TZ has for successful therapy MDR/XDR and TDR MT infections [123] (Abbate et al., 2012). However, it should be noted that in all probability, TZ will be useful for the therapy of any intracellular infection such recurrent Staphylococcal pulmonary and bone marrow infections [61] (Ordway et al., 2002), as well as Chagas disease, leishmania, and even malaria, given that its precursor PZN CPZ has already been shown to kill the infectious agent [124-128].

Conclusion

The gross structural similarities such as methylene blue and the Phenothiazine (PZN) CNS agents, they may be less apparent in second- and third-generation agents, such as the SSRIs. The chemical links do not explain entirely the antimicrobial activities of the non-antibiotic derivatives, although, the in vivo reduction of methylene blue and its incorporation as the neutral leucobase by tubercles hints at the similarity. The improvement in selectivity and action of neuroleptic drugs progressing from Chlorpromazine (CPZ) to Thioridazine (TZ) and the thioxanthenes allowed effective therapy at lower doses with fewer side effects. A similar pattern has been shown with the antidepressants, from tricyclics to SSRIs. The discovery that many of these agents are also antimicrobial thus offers the new approaches and templates for novel drug design-in antibacterial chemotherapy. The adjunctive use of suitable CNS agents alongside conventional antibacterial agents in order to circumvent clinical resistance is likely to be similarly effective. The PZNs have broad antimicrobial activity that is expressed against intracellular antibiotic-resistant bacteria such as Mtb, S. aureus and antibiotic-resistant protozoa like P. falciparum. The PZNs inhibit ABC type efflux pumps that account for the antibiotic resistance of the organism.

Because PZNs inhibit calcium binding to calmodulin or calmodulin-type proteins, much in the manner of the calcium channel verapamil, they may also affect all verapamil-sensitive efflux pumps. The antimicrobial activity of TZ, is equal to the more toxic CPZ. Therefore, the relatively mild TZ has potential for the therapy of problematic antibiotic-resistant intracellular infections. The PZNs may also be useful as inhibitors of efflux pumps responsible for the antibiotic resistance of many microbes. In contrast to atypical antipsychotics, which increase the risk of metabolic syndrome and diabetes mellitus and thus secondary infections including TB, particularly MDR, PZNs may, in certain patients, useful for the treatment of TB infections. They can be of particular use in areas with a high prevalence of MDR-TB where they can be used as an adjunct to conventional therapy during initial treatment. Because the length of such treatment is anticipated to be weeks, side effects associated with chronic TZ therapy are not anticipated. Though the efficacy of PZNs as anti-TB agents especially against phagocytosed bacteria, there are still many unanswered questions. It is therefore necessary to conduct further clinical trials to determine the role of PZNs in the treatment of TB especially, MDR-TB [129-131] finally; it should provide chemotherapy at much lower cost, thus being of considerable potential in aiding less fortunate health economies.

References


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