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Thioridazine: resurrection as an antimicrobial agent?

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The emergence of multiresistant bacterial strains and the continuing burden of infectious disease globally point to the urgent need for novel affordable antimicrobial drugs. Thioridazine is a phenothiazine antipsychotic drug with well-recognized antimicrobial activity, but this property has not been harnessed for clinical use as a result of its central nervous system and cardiac side-effects. The cardiotoxicity of thioridazine has recently been shown to be structurally specific at a molecular level, whereas its antimicrobial properties are shared by a number of phenothiazine analogues. This raises the possibility that its enantiomers or its inactive metabolite, the ring sulphoxide, may act as a lead compound in the future development of antimicrobial drugs to face the new challenges in infectious disease.

Introduction

Phenothiazine drugs, in addition to their antipsychotic properties, have significant antimicrobial activity against a wide variety of intracellular microorganisms [1], as they are concentrated almost 100-fold in macrophages [2] and lung [3].

The prototypical phenothiazine drug, methylene blue (methylthioninium chloride), was shown to be active against *Plasmodium falciparum* by Ehrlich in 1891 [4]. Subsequently developed phenothiazines, such as chlorpromazine, have proven *in vitro* bacteriostatic and bactericidal activity against several microorganisms, including *Mycobacterium tuberculosis*. This antimicrobial potential has not been harnessed to date due to concern over the sedative and extrapyramidal side-effects and the cardiotoxicity of phenothiazine drugs at the plasma concentrations required to achieve bactericidal effects.

Thioridazine (an alkylpiperadine phenothiazine), previously used extensively for its antipsychotic properties, has recently attracted interest as a potential candidate for development as an antimicrobial drug, as it is associated with the lowest risk of extrapyramidal side-effects of the phenothiazine drugs [5]. However, thioridazine can cause cardiac repolarization abnormalities and QTc prolongation at therapeutic doses [6–8] and reports of torsade de pointes [9] are well documented. A series of studies showing increased risk of QTc prolongation [6, 10] and sudden death [11–13] in patients treated with thioridazine has led to a re-evaluation of its use by drug regulatory agencies worldwide, culminating in the

voluntary withdrawal of branded versions of the drug by Novartis in June 2005. Although it is still available in generic form, there has been a marked decline in prescription of thioridazine in developed countries.

The antimicrobial properties of phenothiazine drugs (with particular reference to thioridazine) and recent developments in our understanding of thioridazine cardiotoxicity are reviewed in this article, followed by a discussion of their implications for potential clinical use and further drug development.

The need for new antimicrobial agents

The development of antibiotic therapy has led to a massive reduction in the morbidity and mortality associated with infectious diseases in the developed world. Nevertheless, the incidence of serious nosocomial infections resulting from the emergence of bacterial strains resistant to conventional antibiotics, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci, has significantly increased over the past decade [14]. Although glycopeptide (vancomycin and teicoplanin) and oxazolidinone antibiotics (linezolid) are available for the treatment of MRSA-associated infections, vancomycin-resistant strains are emerging and the cost of these drugs is prohibitive in developing countries.

Mycobacterium tuberculosis currently infects over 2 billion people worldwide and accounts for >1.5 million deaths annually. The global proportion of multidrug resistant (MDR) TB is estimated to be around 1–2% of all cases [15]. The resurgence of tuberculosis amidst the global acquired immunodeficiency syndrome epidemic and the increasing frequency of drug-resistant strains are matters of public health concern worldwide.

Resource-poor developing countries continue to suffer the socioeconomic and health consequences of endemic diseases such as malaria, leishmaniasis and Chagas disease. Over 3 billion people live in regions where malaria is endemic. Malaria is a devastating disease with an annual morbidity of 300–500 million people and annual mortality of over one million [16]. Chloroquine-resistant strains of *P. falciparum,* responsible for the most lethal form of human malaria, are now common in most malaria-endemic regions where artemisinin-based therapies are often unaffordable. Twelve million people are estimated to be infected and 2 million new cases of leishmaniasis occur annually worldwide [17]. Of the population of Latin America, 25% is at risk of acquiring Chagas' disease. Current antitrypanosomal drugs such as nifurtimox are highly toxic, resulting in poor patient compliance.

The emergence of multidrug-resistant bacteria has led to revived interest in the search and development of new antibiotics to add to our existing armamentarium. However, with the spiralling cost of new drug discovery estimated to exceed $750M per new chemical entity [18], there is insufficient economic incentive for the pharmaceutical industry to develop novel drugs to tackle infectious diseases endemic in developing countries. In this context, the development of existing chemical entities with documented antimicrobial activity must be explored in an attempt to bring affordable drugs to the billions of people worldwide afflicted by common endemic infectious diseases.

Antimicrobial activity of thioridazine

MRSA

Thioridazine, in addition to its activity against intracellular methicillin-susceptible *S. aureus* (MSSA) [19], has demonstrable activity against MRSA with minimum inhibitory concentrations (MIC) ranging between 16 and 50 mg l^{-1} [20–22]. Addition of thioridazine at concentrations of 25–50% of its MIC to conventional antibiotics has led to a two-to-eightfold reduction in the MIC of norfloxacin [22] and a reduction in the MIC of oxacillin from >500 mg l^{-1} to 10 mg l^{-1} against some MRSA strains [23]. This is due to inhibition by thioridazine of bacterial efflux pumps which confer antibiotic resistance [22–24]. In addition, at subinhibitory concentrations, thioridazine inhibits the replication of phagocytosed MRSA and causes ultrastructural changes in the cell envelope structure, resulting in bacterial lysis after phagocytosis [21]. The mechanism of action of thioridazine is not fully understood, but the ultrastructural changes are similar to those produced by β-lactam antibiotics, suggesting that inhibition of membrane-bound enzymes may partly be responsible.

Enterococcus species

Multiresistant enterococci have emerged as a cause of serious nosocomial infections over the past decade. These strains produce β-lactamase enzymes conferring resistance to multiple antibiotics, including penicillins, carbapenems and glycopeptides, and also possess multidrug resistance efflux pumps. The finding that methylene blue and two methylated derivatives have bactericidal activity against vancomycin-resistant pathogenic strains of *Enteroccus* species [25] has led to the investigation of thioridazine as a potential antienterococcal antibiotic. Thioridazine inhibits *E. faecalis* and *E. faecium* strains (originating from human infections and animal faecal flora) at a concentration of 16–32 mg l^{-1}, regardless of their antibiotic sensitivity. At subinhibitory

concentrations, thioridazine has shown synergistic effects when combined with vancomycin or ampicillin, by a mechanism unrelated to P-glycoprotein-mediated multidrug resistance [26].

Mycobacterium tuberculosis

Thioridazine has significant *in vitro* activity against susceptible and multidrug-resistant strains of *M. tuberculosis*, with reported MIC varying from 6 to 32 mg l^{-1} [27–29]. It also acts synergistically with some first-line antituberculous drugs [30] and may allow reduced doses of these drugs to be used. *In vitro* experiments using THP-1 macrophage cell lines and human peripheral monocyte-derived macrophages infected with *M. tuberculosis* have shown that the minimum bactericidal concentration of thioridazine is as low as 0.1 mg l^{-1}, with complete killing occurring within 3 days of infection [29]. Phenothiazines affect a number of key mycobacterial targets [31–33]. They bind to and inactivate calmodulin, a calcium transport protein which is a vital constituent of the cell wall envelope of mycobacteria [34–36]. Development of resistance to thioridazine is unlikely, as mutations affecting mycobacterial calcium flux would affect the viability of the organism. Genomic analysis of *M. tuberculosis* led to identification of type II nicotinamide adenine dinucleotide (NADH) dehydrogenase as a key enzyme for bacterial growth under aerobic conditions and a specific target for drug action, as human mitochondria only use type I NADH dehydrogenase. The antituberculous activity of phenothiazines appears to be partially due to specific inhibition of type II NADH dehydrogenase, as determined by NADH:menaquinone oxidoreductase activity [37].

Plasmodium falciparum

Since the initial description of its activity against *P. falciparum* by Ehrlich [38], methylene blue has been shown to inhibit *P. falciparum* glutathione reductase. Evaluation of methylene blue in combination with chloroquine in children with uncomplicated *P. falciparum* malaria in sub-Saharan Africa has confirmed its antimalarial effects [39]. Newer phenothiazines have also been shown to have *in vitro* activity against *P. falciparum* [40–42]. In a preliminary screening study of existing chemical entities against two strains of *P. falciparum,* thioridazine inhibited growth of *P. falciparum* within clinically achievable therapeutic plasma concentrations of thioridazine (effective 50% inhibitory concentrations, EC_{50}, of 1.9 and 2.6 μM) [43].

Trypanosoma cruzi

Trypathioninone plays a prominent role in the redox defences of pathogenic Trypanosoma species. Phenothiazines inhibit two key trypanosomal enzymes, trypanothione reductase [44] and dihydrolipoamide dehydrogenase [45, 46], and also induce mitochondrial disruption in epimastigote and tripomastigote forms by formation of cationic free radicals through the peroxidase/H_2O_2 sytem [47]. In mice with experimental Chagas' disease, thioridazine significantly improved survival and cardiac function in the acute phase [48, 49] and chronic phase of the disease [50].

Other parasites

Thioridazine has been shown to be the most active phenothiazine agent against *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* [19] and *M. avium in vitro* [51]. Although thioridazine has not been tested specifically, other phenothiazines also appear to be active against *Leishmania* species [52, 53], *Schistosoma mansoni* and *Trypanosoma brucei* and *gambiense* [54].

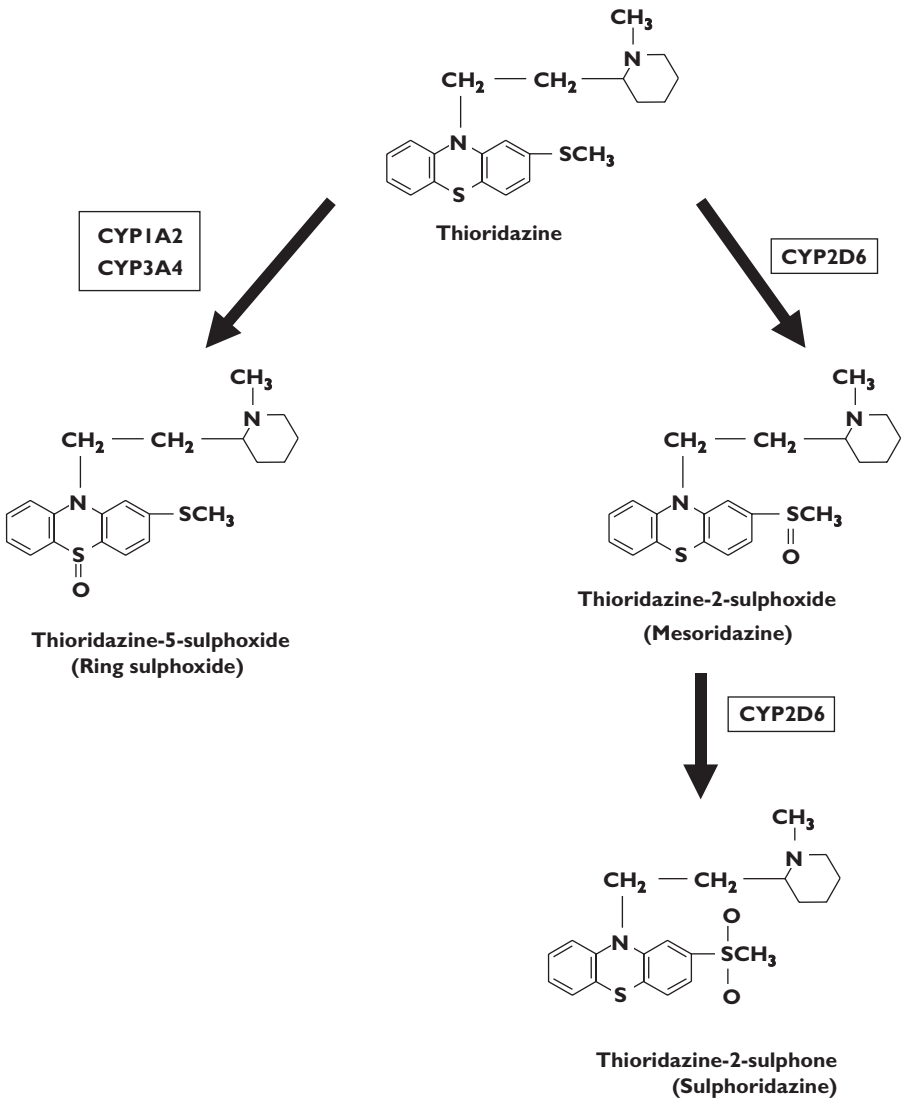
Clinical pharmacology of thioridazine

Following oral administration, thioridazine is rapidly absorbed, with peak plasma concentrations occurring within 2–3 h [8]. Plasma concentrations of thioridazine and its metabolites achieved in clinical use show wide interindividual variability and are affected by factors such as age, smoking and genetic polymorphisms in drug-metabolizing enzymes [55–57]. Thioridazine is widely distributed to tissues throughout the body.

Thioridazine is metabolized to mesoridazine (thioridazine-2-sulphoxide), which undergoes further 2-oxidation to sulphoridazine (thioridazine-2-sulphone). Thioridazine also undergoes 5-oxidation to the ring sulphoxide (thioridazine-5-sulphoxide) (Figure 1) [8]. Studies using human liver microsomes suggest that the metabolism of thioridazine to mesoridazine and sulphoridazine is catalysed mainly by CYP2D6, but CYP1A2 and CYP3A4 are the main isoforms involved in the formation of the ring sulphoxide [58]. Drug–drug interactions may therefore occur when drugs which inhibit or induce CYP2D6, CYP1A2 or CYP3A4 are used concomitantly. Smokers have lower plasma concentrations of thioridazine, mesoridazine and sulphoridazine compared with nonsmokers [56, 57]. Patients who are genetically poor metabolizers of CYP2D6 or take drugs which are potent inhibitors of CYP2D6 have higher plasma concentrations of thioridazine and the ring sulphoxide due to inhibition of metabolism to mesoridazine [55, 57].

The therapeutic thioridazine plasma concentration for its antipsychotic efficacy is 0.5–1.0 mg l^{-1}. Thioridazine exhibits linear kinetics within the dose range used clinically. The plasma concentrations of thioridazine and its

Figure 1
Metabolic pathway of thioridazine in man



metabolites per mg of thioridazine given orally as a single dose and at steady state after chronic once-daily dosing are shown in Table 1. The mean half-life of thioridazine is 6.5 h in healthy volunteers after single dosing, but may be significantly prolonged to 40 h in the elderly. The ring sulphoxide is the main metabolite in chronically treated patients due to its longer elimination half-life (Table 1) [8].

Thioridazine, mesoridazine and sulphoridazine are all potent D2 receptor blocking agents in rat and rabbit striatal membranes [59, 60], suggesting that they all contribute to the antipsychotic effects and the extrapyramidal side-effects of thioridazine. However, the ring sulphoxide metabolite (thioridazine-5-sulphoxide) is thought not to have antipsychotic activity [61].

Thioridazine is administered clinically as a 50 : 50 racemic mixture of its two enantiomers (R) and (S)-thioridazine. In isolated rat brain preparations (R)-thioridazine has 2.7 times higher affinity than (S)-thioridazine for D2 receptors, and acute administration of (R)-thioridazine to rats induced slightly more catalepsy than (S)-thioridazine and appeared to be more toxic at large doses [62].

Cardiotoxicity of thioridazine

The cardiac effects of thioridazine are related to the concentration-dependent blockade of the cardiac delayed inward rectifier potassium channel (I_{kr}) [63, 64]. Mesoridazine has similar concentration-dependent I_{kr} channel blocking activity to the parent drug [65]. Thioridazine and, more recently, mesoridazine have been shown to prolong the QTc interval following single-dose administration to healthy volunteers [8, 66]. It has been postulated that the cardiotoxic effects

Table 1
Pharmacokinetic parameters of thioridazine and metabolites

	Thioridazine	Mesoridazine	Sulphoridazine	Ring sulphoxide
Mean half-life after single 50-mg dose (h)*	6.5	8.7	9.6	18.4
Mean peak plasma concentration per mg thioridazine after single 50-mg dose ($\mu g\, l^{-1}\, mg^{-1}$)*	2.0	5.0	0.9	2.1
Range of steady-state plasma concentration per mg thioridazine after once daily dosing ($\mu g\, l^{-1}\, mg^{-1}$)†	0.4–8.8	0.6–13.4	0.2–3.3	0.2–41.0

**Values for half-life and peak concentrations after single dosing are derived from Hartigan-Go* et al. *[8]. †Values for thioridazine, mesoridazine and sulforidazine are derived from Berecz* et al. *[56], and for the ring sulphoxide from Thanacoody* et al. *[57].*

of thioridazine may be due to the ring sulphoxide as a result of the reported cardiotoxic effects in the isolated perfused rat heart [67] and in dogs [68]. Conflicting animal studies [68, 69] and findings of recent studies in patients treated with thioridazine and in healthy volunteers receiving mesoridazine have cast doubt on this hypothesis [57, 66]. The QTc interval at steady state was shown to be significantly correlated with the plasma concentrations of thioridazine and its 2-oxidation metabolites (mesoridazine and sulphoridazine), but not with the 5-oxidation metabolite (ring sulphoxide) [57]. The effect of the ring sulphoxide on I_{kr} channels has not been studied and these findings should be considered preliminary. Nevertheless, recent understanding of the mechanism of thioridazine I_{kr} blockade at the molecular level may explain why the ring sulphoxide does not appear to prolong the QTc interval in patients on thioridazine and may pave the way to derivatives of thioridazine which do not cause this side-effect.

Human *ether-a-go-go*-related gene (*hERG*) potassium (K^+) channels mediate the rapidly activating delayed rectifier K^+ current (I_{Kr}), which plays a key role in repolarization of the ventricular action potential. This is not involved in the electrophysiology of the rat and studies in the isolated perfused rat heart cannot therefore be extrapolated to man. In man, four hERG subunits assemble as a tetramer to form the ion channels that conduct the I_{kr} current. They consist of voltage sensor regions (S1–S4) and pore regions (S5–S6). The pore-forming unit of the I_{kr} channel contains a high-affinity drug binding site, of which aromatic amino acids present in the inner (S6) helices are key components [70]. It has been demonstrated that thioridazine causes I_{kr} blockade as a result of binding to the S6 helix amino acid residue F656 [71]. Single mutation F656A at this site leads to almost complete abolition of thioridazine-induced I_{kr} blockade [71].

As a result of the specificity of thioridazine-induced blockade, it is possible that 5-sulphoxidation leads to molecular changes, which reduce the affinity of the ring sulphoxide for that binding site.

Potential antimicrobial use of thioridazine

Thioridazine shows most promise for the adjunctive treatment of infections caused by multiresistant strains of intracellular organisms such as *M. tuberculosis* and *P. falciparum* in resource-poor countries [40]. Although the *in vitro* MIC against *M. tuberculosis* is high, killing of phagocytosed mycobacteria in macrophages occurs at a thioridazine concentration of 0.1 mg l^{-1} (which is clinically achievable using oral doses as low as 10–20% of those used in psychiatry) because of the ability of macrophages to concentrate phenothiazines [29]. Plasma thioridazine concentrations corresponding to the *in vitro* inhibitory concentrations of 0.75–1.0 mg l^{-1} for two strains of *P. falciparum* [43] can be achieved using oral doses used in psychiatric practice. Further evaluation is required in animal models of infection to determine whether clinical efficacy can be achieved *in vivo* prior to the conduct of clinical trials in man. The potential use of low-dose thioridazine as antimycobacterial prophylaxis in HIV-infected patients in areas with a high prevalence of HIV seropositivity may also be explored.

With the advent of newer antipsychotic drugs with a better safety profile, the use of thioridazine in psychiatry has declined in developed countries as the risk–benefit ratio is considered to be unfavourable. However, large epidemiological studies have shown that the risk of cardiotoxicity is small, with <20 episodes of torsade de pointes or sudden death potentially attributable to use of thioridazine occurring per 10 000 patient-years of treat-

ment [12, 72]. In areas with a high prevalence of drug-resistant malaria and TB, the potential benefits outweigh the risks and conduct of clinical trials and subsequent clinical use of thioridazine may be justified if clinical efficacy is demonstrated. Investigation of methylene blue in combination with chloroquine in children with malaria in an area of high chloroquine resistance demonstrates that such clinical trials are both ethically justifiable and feasible [39].

Although thioridazine is active against resistant strains of *S. aureus* and enterococci, the plasma concentrations required for bactericidal activity as a single agent are not clinically achievable without intolerable side-effects and therefore preclude its use for serious bacteraemic infections with these organisms. Nevertheless, the use of thioridazine in combination with other antibiotics for its synergistic effects deserves some further investigation, as higher concentrations of thioridazine may be achieved in infected tissues despite plasma concentrations lower than the MIC seen *in vitro*.

Potential avenues for drug development

Novel derivatives of thioridazine that have reduced affinity for the I_{kr} channel and dopamine receptors but have higher potency as an antimicrobial have potential to meet the new challenges in infectious disease.

This is theoretically possible since a number of phenothiazine agents and various derivatives of thioridazine exhibit antimicrobial activity *in vitro,* although the metabolites of thioridazine have not specifically been investigated. In preliminary studies, novel derivatives of thioridazine enhance the bactericidal activity of phagocytosed *M. tuberculosis* at concentrations of 0.1 mg l^{-1} [73]. Phenothiazine analogues with greater potency have also been characterized and shown to suppress *M. tuberculosis* growth in a mouse model of acute infection [37]. The S-enantiomer of thioridazine and other phenothiazine isomers has also recently been demonstrated to have antimicrobial properties with reduced central nervous system effects [74]. Different EC_{50} values were obtained for analogues of thioridazine when tested against two strains of *P. falciparum*, raising hope that structural optimization may be possible [43]. The antimicrobial properties of thioridazine therefore appear to lack the same structural specificity required for its cardiotoxic effects.

As thioridazine-5-sulphoxide does not appear to have antipsychotic effects which is the result of binding to dopaminergic receptors in the brain, it is unlikely to cause troublesome sedating and extrapyramidal side-effects and could therefore serve as a lead compound in the development of more potent and safer derivatives for clinical use. In addition, thioridazine-5-sulphoxide exist as two pairs of enantiomers in equal concentrations, known as thioridazine-5-sulphoxide fast-eluting (FE) and slow-eluting (SE), based on their separation properties by chromatography [75]. The characteristics of these enantiomers are unknown, but they are likely to have differing affinities for the D2 receptor and I_{kr} ion channels, offering another potential avenue for optimization of the structure–activity relationship.

Conclusions

Thioridazine may have lost its shine as an antipsychotic, but its potential as an antimicrobial can no longer be ignored, least of all in the vast areas of the world plagued by endemic infectious diseases. Further 'proof of concept' studies are required to investigate the binding of the ring sulphoxide and its enantiomers to the I_{kr} channel and dopamine receptors *in vitro*. Partnerships between academia and the pharmaceutical industry offer the best chance of developing a safe, effective and affordable drug using an existing chemical entity as a lead compound.

Competing interests: None to declare.

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