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Review

Antimicrobial Activity of Phenothiazines

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Abstract. *Multidrug-resistant Mycobacterium tuberculosis (MDRTB) and antibiotic-resistant Plasmodium falciparum are the major global lethal infections accounting for over 4 million deaths per year. Methicillin-resistant Staphylococcus aureus (MRSA) is the major global nosocomial infection and resistance to vancomycin is evident and may become common. This review provides the scientific and medical basis that support the use of one particular group of compounds, the phenothiazines, and in particular thioridazine, for the management of the above antibiotic-resistant infections. Because thioridazine, a relatively mild neuroleptic as compared to its parental compound chlorpromazine, kills intracellular MDRTB and MRSA at clinical concentrations, its use for the management of these infections may be considered. The review also discusses the activity of phenothiazines against protozoa and parasites, the mechanisms by which phenothiazines promote their antimicrobial effects, their potential for regulating efflux pumps that are a cause for mono or multidrug resistance, as well as their potential for the therapy of problematic infections caused by bacteria that have acquired plasmid-antibiotic-resistant genes.*

History

The antimicrobial activity of phenothiazines has been known since the time of Paul Ehrlich (1). 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 synthesised and used effectively for the therapy of psychosis (2). The first such compound, chlorpromazine (CPZ), was made available in 1953 by Rhone-Poulenc (3) 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 (4), and whatever antimicrobial activity reported was essentially one that was produced *in vitro* and at clinically irrelevant concentrations (4, 5), CPZ or other phenothiazines were not seriously considered as potential sources of new antibiotics, even when they were shown to have desired antimicrobial effects *in vivo* (2). However, the global increase of MDRTB, quinidine-resistant malaria, nosocomial MRSA infections, *etc.*, primarily in countries that cannot afford available antibiotics, notwithstanding the problem of resistance, dictates that phenothiazines be now seriously considered where other drugs have failed.

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Antimicrobial Activity of Phenothiazines

The antibacterial properties of phenothiazines may be summarised as follows: gram-positive cocci (6-8), Mycobacteria (2, 4, 9, 10-12) and some gram-negative rods, such as *Shigella* spp., are more susceptible to a number of phenothiazines as opposed to gram-negative rods such as *Escherichia coli* (6) and *Salmonella* spp. (13) in general, with MIC's that range from 20 to 30 μg/ml for the "susceptible group", and in excess of 100 μg/ for the "resistant group".

It is important to note that, regardless of the method employed for assaying the activity of the phenothiazine, all of the activities take place at concentrations that greatly exceed the highest plasma concentration achievable, namely 0.5 μg/ml (6). Although this data at face value suggests that the antibacterial use of these compounds is not feasible, smaller concentrations of phenothiazines do enhance the

activity of antibiotics to which the bacterium is susceptible (1, 14-16), even when it is resistant to the antibiotic (8). The latter observations suggest that these compounds may serve as adjuvants whenever there is a need to reduce the dose of a given antibiotic or render an antibiotic-resistant infection susceptible to the antibiotic (1, 14). Nevertheless, although the concentrations of the phenothiazines 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 phenothiazines as antibacterial agents or as enhancers of antibiotic activity lies in their ability to kill phagocytosed bacteria. *Mycobacterium tuberculosis* and *Staphylococcus aureus*, that have been phagocytosed by macrophages that by themselves have little killing activity of their own, are effectively killed (6, 7, 12, 17). 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 (6, 7, 12). Killing apparently is the result of the ability of the macrophage to concentrate the phenothiazine to a level comparable to a minimal bactericidal concentration (6, 7, 12, 17), a property previously shown for tissues and organs that are rich in macrophages (18, 19).

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 *in vitro* or *ex vivo* (*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 that 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 (20) in febrile neutropenia accompanying cancer chemotherapy (21), and other diseases presenting with neutropenia and weak granulocyte functions, *i.e.*, glycogen storage disease type Ib (22), whose basis for recurrence lies in the intracellular location of the organism that is not killed by the macrophagic cell.

Phenothiazines are known to alter the morphology of bacteria when the concentration of the phenothiazines is below that which inhibits the cell's replication (1). The alterations are specifically related to the species, such that the phenothiazines causes filamentation of *E. coli* (23) and *Salmonella thyphimurium* (13) and cluster formation of *S. aureus* resulting from unseparated daughter cells (1, 24). It is interesting to note that these respective responses to the phenothiazines are very similar to those evoked by sub-inhibitory concentrations of a beta-lactam (25). 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 (25- 27), phenothiazines 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 (27), as well as by physical conditions such as release from hydrostatic pressure (28) and by growth conditions (29), the filamentation of a gram-negative rod caused by sub-inhibitory concentrations of a phenothiazine may not involve a direct effect on the PBP itself.

Phenothiazines have been shown to reduce the adherence of gram-negative bacteria to epithelial cells (30, 31). The phenothiazine promethazine prevents the recurrence of pylonephritis caused by *E. coli* in pediatric subjects (32) and, because the concentration of this phenothiazine 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 phenothiazine has on the adherence of *E. coli* to the epithelium of the urinary bladder, the latter being a pre-requisite for eventual development of pylonephritis. Although the effects of a phenothiazine on structures of the gram-negative bacteria needed for adherence such as pili (33), 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 (34), have not yet been fully studied, it seems probable that phenothiazines do inhibit adherence by inhibiting pili formation, much as is true with low concentrations of antibiotics (35), 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 phenothiazines described most probably account for cures of bacterial infections treated with phenothiazines. Mice infected with *Salmonella typhimurium* can be cured with trifluoperazine (36) or fluphenazine (37), by a combination of trimethoprim and trimeprazine (38). 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 (39); and mice infected with Mycobacteria could be cured with methdilazine (40).

The curative effects of chlorpromazine on humans presenting with bacterial infections are also known and have been reviewed elsewhere (1, 2, 4). Although there currently exists sufficient support for the use of phenothiazines, especially the far less toxic thioridazine, 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 has been known for over a century (41). 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 (42). 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 (43), there is a dire need for effective antimalarials. CPZ and other phenothiazines are known to have *in vitro* activity against *Plamodium falciparum* (44-46). CPZ effectively cures the Aortus monkey of a *P. falciparum* infection and reverses resistance to chloroquine (47). However, since not all Plasmodia resistant to chloriquine can be made sensitive to the antimalarial (44), this may indicate strain- and/or species- based differences with respect to the modulation of antimalarial resistance by the phenothiazine. 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 (41).

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 (48). Therapy for this infection is problematic since the side-effects produced by conventional drugs are considerable (48) and, as is the case with bacterial and malarial infections, resistance to drugs such as antimonials is quite common today (49). Phenothiazines and acridines have long been known to have activity against *Leishmania*-causing parasites (50, 51), however, the concentrations needed for this activity are either toxic or clinically irrelevant (52). Although topical application of CPZ has been reported to effectively cure cutaneous leishmania (53), others claim otherwise (54). Nevertheless, because *Leishmania* is an intracellular parasite, CPZ will kill the intracellular organism (55). TZ will probably prove to be as effective against intracellular species of *Leishmania*.

Antiviral Activity

CPZ has been shown to have activity against a broad gamut of viruses (2). As early as 1971, it was shown to inhibit the modification of host cell membranes caused by herpes simplex (56). Thereafter, it was shown to inhibit the growth of TBEV (57), inhibit the activity of hepatitis B DNA (58), lyse a number of viruses (59), inhibit the conjugal transfer of R and F'lac plasmids (60), inhibit the budding of measles virus (61) and Sindbis and vesicular stomatatitis virus (62), inhibit the replication of influenza virus (63), SV40 (64), arenavirus (65) and HIV (66), block infection of B lymphocytes by human herpes virus (67,68) and infection of tissue culture cells by JC virus (JCV) (69). 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 (66, 70), it inhibits calcium-dependent events that take place at the plasma membrane and which are required for entry of the virus *via* endocytosis (71), and it inhibits the replication of DNA primarily by intercalating between the bases (72,73).

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 phenothiazine methylene blue can be substantially enhanced when the presentation of this compound to a virus takes place under photo-activation (74). 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 (75), plasma, platelet concentrates and coagulation factors (76,77), and cryoprecipitates and cryosupernatants (78). The successful use of photo-activated methylene blue has prompted consideration that this approach may have some value for managing problematic viral infections (74).

Antiprion Activity

CPZ and acridines have been shown to eliminate the presence of prions of infected mouse neuroblastoma cells chronically infected with the prion PrP(Sc). (79). These results were employed for effective but temporary therapy of two young women presenting with nvCJD (2). The theoretical mechanism by which phenothiazines destroy intracellular prions and the suggested treatment of the prion infections have been reviewed elsewhere (2, 80).

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 (81, 82). Antibiotic-resistant genes present in plasmid-containing bacteria can cause serious therapeutic failure (83 -86) or

manifest as a consequence of the selection of the resistant plasmid-containing strain (87). 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, phenothiazines are known to promote the elimination of plasmids from infected bacteria (88 - 92). The therapeutic aspects of the antiplasmid effects of phenothiazines and the mechanism by which the effect takes place are beyond the scope of the present review and will be reviewed elsewhere.

Efflux Pumps and Effects of Phenothiazines

To date, all micro-organisms so far studied have been shown to have a number of efflux pumps which, by extrusion of obnoxious compounds, afford protection. Although the efflux pumps of bacteria, fungi, protozoa and parasites have been extensively reviewed elsewhere (93), the effects of phenothiazines on these efflux pumps do lower antibiotic resistance and, in certain cases, account for a complete reversal of antibiotic resistance. Furthermore, the role of efflux pumps as a major mechanism for intrinsic, acquired or adaptive antibiotic resistance merit that these units receive some consideration in this general review.

Prolonged exposure of cancer cells to a single chemotoxic agent results in the cells becoming resistant to that agent. Resistance is not mediated by mutation, but rather by the induction of energy-dependent efflux pumps that extrude not only the agent to which they had been exposed, but also other drugs. These efflux pumps are termed multidrug-resistant (mdr) and intensive study has shown the mdr nature of these pumps is caused by transmembrane xenobiotic transport molecules that belong to the super family of ATP-binding cassette transporters (94). The main characteristic of these mdr efflux pumps is that the energy required for transport of one or more unrelated molecules is derived from the activity of calcium-dependent ATPase, and this energy activates a plasma membrane protein p-glycoprotein (Pgp), which is responsible for binding and extruding the drug (95). A large number of Pgp transporter proteins have been described for mammalian cells, some of which are similar to those present in micro-organisms (96). Mdr efflux pumps are generally inhibited by the calcium channel blocker verapamil (97) and, because phenothiazines inhibit the binding of calcium to calmodulin (98) or calmodulin-type proteins (99), they have been considered as potential inhibitors of mdr efflux pumps (100). Phenothiazines have been shown to inhibit the efflux pumps that account for antibiotic resistance in cancer cells (100) and bacteria (8, 100, 101) and reverse antibiotic resistance of bacteria (8, 101, 102). Because verapamil inhibits the efflux pumps of yeast, protozoa (103-106,) and parasites (107) as well as reversing resistance of Plasmodia to chloroquine (104), it is anticipated that phenothiazines will also inhibit these and other mdr efflux pumps as well.

Conclusion

Phenothiazines have broad antimicrobial activity that is expressed against intracellular antibiotic-resistant bacteria such as *M. tuberculosis*, *S. aureus* and antibiotic-resistant protozoa such as *P. falciparum*, at concentrations that are clinically relevant. Whenever studied, phenothiazines inhibit ABC type efflux pumps that account for the antibiotic resistance of the organism. Because phenothiazines 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 thioridazine, whenever studied, is equal to the more toxic chlorpromazine. Therefore, the relatively mild thioridazine has potential for the therapy of problematic antibiotic-resistant intracellular infections. Moreover, this and other phenothiazines may also be useful as inhibitors of efflux pumps responsible for the antibiotic resistance of many micro-organisms.

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